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QUALITY CONTROL REQUIREMENTS
FOR
PEST BIOLOGICAL CONTROL AGENTS

Alberta
ENVIRONMENTAL CENTRE

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QUALITY CONTROL REQUIREMENTS
FOR
PEST BIOLOGICAL CONTROL AGENTS

CANADIANA

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ABSTRACT

The predatory mites *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius cucumeris* Oudemans, and the parasitoid wasp *Encarsia formosa* Gahan are used in Alberta greenhouses in biological control programs to control spider mite, thrips, and whiteflies respectively. Following persistent complaints from growers in 1989 and 1990 about poor quality and lack of efficacy, several shipments from three commercial suppliers were evaluated in 1991. In the case of shipments of *P. persimilis* and *A. cucumeris*, there was significant divergence from stated contents both among suppliers and among batches. In laboratory tests for survival and fecundity, predator mites from most batches performed poorly. Emergence of *E. formosa* from parasitized whitefly pupae was generally low and less than 60 percent. Samples from the shipments were examined for the presence of microorganisms that might cause disease. Several of possible significance were found, including rickettsia, microsporidia, and virus, all previously undescribed. The poor performance of some shipments is discussed in relation to possible causes such as shipping stress, starvation, improper storage, and disease.

1 INTRODUCTION

The greenhouse industry in Alberta is comprised of approximately 18 ha of vegetable crops, 46 ha of ornamentals, and 4 ha of tree seedlings, with a total value of $42.3 million (M. Mohyuddin, personal communication). The major vegetable crop is cucumber, followed by tomato. There is some interest in pepper and lettuce but no significant area as yet. Common pests in vegetable crops are spider mite, whitefly, thrips, and aphids. The most common ornamental crops are bedding plants, followed by poinsettia, chrysanthemum, and tropicals. The same major pests are present on these crops, and pest control is generally required. It is not possible to grow most crops profitably without some degree of pest control. Traditionally, chemical pesticides have been used for this purpose, but the choices have become increasingly limited, and the pests are often resistant to those materials legally available. In 1981, two biological control agents became commercially available in Alberta from Applied Bio-Nomics in Sidney, British Columbia. These were the predatory mite *Phytosieulus persimilis* Athias-Henriot for spider mite (Plate 1, Fig. 1), and the parasitoid wasp *Encarsia formosa* Gahan for whitefly (Plate 2, Fig. 2). *Phytoseiulus persimilis* were shipped on leaves with their mite host, *Tetranychus urticae* Koch. Initially, *E. formosa* were glued to tin foil as black, parasitised whitefly pupae on tobacco leaves, and later as parasitized pupae on paper cards (Plate 2, Fig. 3). Other biologicals were brought in to control various pests in interior plantscapes. Over 80% of cucumber growers initially adopted biological controls, and all the major conservatories, a testimony to the success of the method. In 1989, supplies from two European sources entered the Alberta market. As no plant material could be imported, *P. persimilis* shipped in from Europe were packaged on vermiculite. Applied Bio-Nomics also began offering this distribution method as an alternative to leaves. Periodically, *P. persimilis* and other biologicals were exchanged among suppliers to meet shortages caused by demand or rearing problems.

In 1989, numerous complaints were received from the vegetable growers in southern Alberta about *P. persimilis*, particularly those shipped on vermiculite. Insufficient numbers were present in the containers to control the spider mites. Although improvement of container design and the delivery system increased *P. persimilis* survival, adequate spider mite control was still not achieved. In 1990, trials were run at the Alberta Environmental Centre (AEC) to assess health and performance of the predators. Percentage live and with eggs on arrival, daily egg production of females, and survival over a seven day period were monitored. Mites were obtained from one Canadian and two European sources. Five batches from one supplier were sent on leaves, and the rest on vermiculite, for a total of 16 shipments from the three sources. All suppliers claimed that predators were healthy when shipped and that subsequent problems could be attributed to post-shipment handling. To evaluate this claim, performance of mites in recent shipments was compared with individuals collected from earlier shipments to commercial greenhouses and subsequently reared at AEC. These AEC predators laid more eggs (4.4/day) and lived longer (6.5 days). Comparable figures for the commercial suppliers for eggs laid/day were (on leaves) 2.3, (on vermiculite) 2.97, 0.8, and 0.2 eggs/day, with a lifespan of (on leaves) 4.7, (on vermiculite) 5.8, 3.5, and 3.3 days (K. Girard, M. Steiner, unpublished data). Some predators (all suppliers) were observed with a white discolouration in the abdomen (Plate 1, Figs. 2, 3, see also Plate 3, Fig. 1). Mites in later shipments increasingly showed abnormal body discolourations. Complaints continued to come in from growers in following seasons, only now these included *E. formosa,* commonly used for whitefly control, and *Amblyseius cucumeris* Oudemans, a newly available thrips predator. Many growers became frustrated and returned to using chemical pesticides. The poor performance of imported mites may have been a result of shipping stress, lack of food during transit, disease, or a combination of these factors. In this study, we examine the performance of all three biological control agents.

The objectives of this study were as follows:

1) To determine the parameters necessary for establishing the fitness of the predatory mites *A. cucumeris* and *P. persimilis*, and the parasitoid *E. formosa*, and to apply these parameters to commercial supplies.
2) To establish techniques for determining disease incidence in commercial supplies of *A. cucumeris, P. persimilis*, and *E. formosa.*
3) To identify, as far as possible, any disease organisms present in commercial supplies of *A. cucumeris, P. persimilis*, and *E. formosa.*
4) To determine whether the number of live *A. cucumeris, P. persimilis*, and *E. formosa* received is consistent with the stated contents.

2 LITERATURE REVIEW

Phytoseiulus persimilis, also identified as *P. riegeli* Dosse and *Amblyseius tardi* (Lombardini), was first described from Algeria (Athias-Henriot 1957). The same organism was identified as *P. riegeli* from Chile (Dosse 1958), and *A. tardi* from Sicily (Lombardini 1959). Most present commercial stocks appear to originate with the Chilean material. Early investigations of this predator as a biological control agent for spider mites were conducted by Chant (1957, 1961), Bravenboer & Dosse (1962), Bravenboer (1963, 1965), Vogel (1963), Hussey (1964), Hussey & Parr (1965), Oatman (1965), Oatman & McMurtry (1967), Oatman *et al.* (1967), Ragusa (1965), and Mori & Chant (1966). It has been widely used in both greenhouse and field crops and is considered a very effective predator. Life histories were described by Dosse (1958), Kennett & Caltagirone (1968), Laing (1968), Hessein (1976), Takafuji & Chant (1976), and Amano & Chant (1978), and the information is summarized in Table 1. Rearing methods were described by Scopes (1968), Theaker & Tonks (1977), and Fournier *et al.* (1985); however, most producers now consider rearing methods proprietary information. As far as we can ascertain, most cultures are raised on green beans with two-spotted spider mite, *Tetranychus urticae* Koch, as food.

Amblyseius cucumeris was first described from The Netherlands (Oudemans 1930). It has also been described from England, Australia, Canada, the United States, and Egypt under various synonyms. Life histories were detailed by El-Badry & Zaher (1961), Kolodochka (1985), and Gillespie & Ramey (1988), and the information is summarized in Table 2. Rearing information was detailed by Schliesske (1981), and by Ramakers & van Lieberg (1982) for a related species, *A. mckenziei* Sch. & Pr. (= *A. barkeri* (Hughes)). Use of *A. cucumeris* as a biological control agent against thrips is described by de Klerk & Ramakers (1986), Ravensberg & Altena (1987), Lindhagen & Nedstam (1988), and Gillespie (1989). *Amblyseius cucumeris* is polyphagous. Its diet may include cyclamen mite, spider mites, thrips, honeydew, and pollen. At present *A. cucumeris* is reared commercially on bran, with bran mites (*Tyrophagus* and *Acarus* spp.) provided as food. *Amblyseius cucumeris* is shipped either on the bran rearing medium, which is sprinkled directly onto plants, or in bran-filled paper bags which are hung on plants. The bags constitute a small breeding unit. A small hole in the bag allows slow release of predators.

Encarsia formosa was first described from Ohio in 1924 and found in England in 1926. Its origin is unknown. Speyer described its life history and developed methods for its use as a biological control agent (Speyer 1927, 1929). It was also found to be present in greenhouses in Canada. Life history parameters have been described by Burnett (1949), Madueke (1979), Vet *et al.* (1980), Christochowitz *et al.* (1981), van der Laan *et al.* (1982), and Vianen & van Lenteren (1986). Commercial use has been detailed by many authors, including McLeod (1938), Hussey & Bravenboer (1971), Gould *et al.* (1975), Lindquist (1976), and Stenseth & Aase (1983). Most commercial operations rear *E. formosa* on tobacco with greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), as its host. Black, parasitized whitefly pupae are washed off the leaves, extracted, and glued onto cards. The cards are hung on plants. When mature, the wasp escapes through a small, round exit hole it chews in the pupal case of the host.

Poe & Enns (1970) raised a concern about the effects of inbreeding on closed populations. Other concerns on the quality of biologicals produced through mass rearing were addressed by several authors in "Advances and Challenges in Insect Rearing" (King & Leppla (eds.) 1984), and by Bigler (1989), van Lenteren (1986, 1991) and Leppla & Fisher (1989). The International Organization for Biological Control (IOBC) was formed in 1958 to promote science in biocontrol. There are 20 different working groups; the global working group "Quality Control of Mass Reared Arthropods" was formed in 1981 and meets annually. In the 1991 proceedings, the stated goals of the group were 1. to develop quality control techniques and methods, 2. to support producers of organisms for biological control in implementing quality control in their mass rearing, and 3. to advise governments and producers when establishing quality control regulations. A preliminary list of criteria for quality control for product control procedures was presented (van Lenteren & Steinberg 1991). This list was amended and supplemented in 1992 (D. Elliott, personal communication). A similar group, the Association of Natural Bio-control Producers, formed in the United States in 1990, is attempting to develop similar guidelines. The quality control procedures described in this report, although developed independently, are very similar to IOBC guidelines.

Diseases caused by pathogens have received little attention in articles on quality control and rearing difficulties, and none at all in relation to field performance of predators and parasites used in biological control of pests in greenhouses. There is general acknowledgement that stress and overcrowding brings out latent diseases (Poe & Enns 1970), and mention of antibiotics that might be used to suppress microsporidiosis. There are several reports of a virus infection in citrus red mite (Beavers & Reed 1972, Bird 1967, Gilmore & Munger 1963, Reed & Desjardins 1978, 1982, Reed & Hall 1972, Shaw *et al.* 1967, Smith *et al.* 1959). Šuťáková & Rüttgen (1978) reported on a mixed infection of virus and rickettsia in *Phytoseiulus persimilis* stock originating from Kiev. Šuťáková (1988) and Šuťáková & Arutunyan (1990) conducted further studies on the rickettsia, which she named *Rickettsiella phytosieuli*, but noted no adverse effects despite other members of this genus usually being highly pathogenic. Hess & Hoy (1982) found two viruses and two types of rickettsia-like organisms (RLO's) in the predatory mite *Typhlodromus (= Metaseiulus) occidentalis*. One RLO was associated with reduced fecundity and pale, thin, translucent mites, and the other with plump females with cream or pink rectal "plugs". Pathologies were associated with dense, crowded laboratory colonies.

A microsporidian parasite was found infecting the predatory mites *Amblyseius cucumeris* and *A. barkeri* (Hughes) in mass culture (Beerling & van der Geest 1991). It also attacked the prey mites *Acarus siro* L. and *Tyrophagus putrescentiae* (Schrank). Weiser (1956) identified the microsporidium *Nosema steinhausi* infecting the storage mite *Tyrophagus noxius*. Various fungi and bacteria infect spider mites, but only one report described infections in predator mites. *Conidiobolus obscurus* (Hall & Dunn) killed *P. persimilis* (van der Geest 1985) by direct spray application but not subsequently by residual infection. No reports were found on diseases of *Encarsia formosa*; however, recent interest in mechanisms of sex ratio determination in invertebrates suggest that rickettsias may at least in part be responsible for predominantly female-biased sex ratios in parasitoids, including *E. formosa* (Stouthamer *et al.* 1990, 1993, Zchori-Fein *et al.* 1992).

On the basis of the published reports, we were anticipating finding primarily rickettsia, microsporidia, and virus diseases. A brief review of the three groups follows.

Rickettsia are minute (0.2-0.6µ), bacteria-like organisms which are generally found within host cells, but occasionally may be found outside the cell or attached to its outer surface. They are Gram-negative, pleomorphic, obligate parasites lacking flagella, and are difficult to rear on artificial media. Like bacteria, they are bound by a cell wall and their metabolic enzyme systems can be inhibited by antibiotic and chemotherapeutic agents. They may be rod-shaped, coccoid, or pleomorphic. Most pathogenic rickettsia infect their host after ingestion. They infect midgut epithelium by entering the cells. Replication is by binary fission and in some instances secondary cells are produced which develop into thick-walled infective stages. Pleomorphic forms make it difficult to follow the life cycle. Šuťáková (1988) described the life cycle of *Rickettsiella phytoseiuli* as comprising six morphologically distinct forms (dense, intermediate, bacterial, giant, crystal-forming, and small dark particles). Crystal formation appears common in rickettsia as well but the significance is not known. Rickettsia often occur in vesicles or vacuoles. *Rickettsiella* spp. are generally pathogens, but *Wolbachia*, another genus of rather loosely-associated species, is seldom pathogenic and may instead be associated with mating incompatibilities and sex ratio changes. Some rickettsia are commensal endocytobionts (organisms which live within the host cell, cause no apparent harm to their host, and are transmitted to successive generations as hereditary particles). There is likely a mutual benefit to both host and rickettsia derived from the symbiotic relationship. Schwemmler (1980) reported that animals freed of symbionts suffer an abnormal accumulation of uric acid and other waste. Symbionts would normally perform the function of breaking down these nitrogenous waste products. Transovarial (within the egg) transmission likely ensures transfer of symbionts between generations. Symbionts are generally transmitted only by the female and have limited invasion capabilities under normal circumstances. If rickettsia are commonly found in every individual they are likely to be symbiotic. Concurrent infections with other pathogens may upset the balance and cause the endocytobiont to become pathogenic.

Individuals may show no obvious symptoms of infection, particularly in the case of *Wolbachia* infections. In contrast, *Rickettsiella* often invade the fat body which may cause a bluish discoloration. Other tissues may also be invaded. There may be various behavioural changes and general sluggishness.

Rickettsia show up as minute, birefringent flecks under dark field and grey flecks under phase contrast light microscopy. They sometimes occur in chains. Rickettsia stain positively with aniline dyes. Giemsa and Macchiavello stains are commonly used on smears of infected tissue. Electron microscopy or immunoassays are needed to confirm the presence of rickettsia.

Little is known about many species of rickettsia. While some appear host-specific, others are not. Vertebrate-invertebrate associations are well known, as in the case of *Rickettsia rickettsii*, the agent for Rocky mountain spotted fever, which is transmitted by ticks to man. Ticks are host to several species of rickettsia; in fact, Šuťáková & Řeháček (1988) were able to rear *R. phytoseiuli* successfully in the tick *Dermacentor reticulatus*. All rickettsia of the tribe Wolbachieae, including the genus *Wolbachia*, are associated only with arthropod hosts.

Microsporidia are minute, unicellular protozoa. All are obligate, intracellular parasites. They are common in both vertebrates and invertebrates, particularly insects. The life cycle is complicated and may vary with host sex, life stage, and age, temperature and other environmental conditions. The taxonomic features used in identification are under constant revision.

Two developmental cycles are recognised: merogony and sporogony. In merogony, stages called meronts divide vegetatively by binary or multiple fission. In the next phase, sporogony, sporonts (similar to meronts but with an electron-dense surface coat) divide to produce sporoblasts that mature into spores. The spores are the infective, resistant stage. Spores may develop singly or in groups surrounded by an envelope, termed the sporophorous vesicle. Although usually oval, they may be spherical, pear-shaped, or tubular. In some hosts two or more spore types of the same species may be found. Spore size varies from 1-20µ (average about 4µ). The mature spore contains a whip-like structure called the polar flagellum, which is attached at the anterior pole of the spore by an anchoring disc and coiled in the posterior part of the spore. Characteristics of the polar flagellum, internal and external structures, wall thickness, number of spores in a sporophorous vesicle, and the number of nuclei in the spore (one or two) are diagnostic for species (Larsson 1988) (see Plate 17, Fig. 2 for generalized diagrammatic representation of a spore). The spores are generally ingested with food or on contaminated material (horizontal transmission), but transovarial (within the egg) or trans-ovum (on the egg surface) transfer (vertical transmission) is not uncommon. Once in the gut of the host, ingested spores are triggered by external stimuli to evert the polar flagellum. The germ plasm in the spore (the sporoplasm) moves through the flagellum under pressure and is injected into the host epithelial cell. Starved hosts are reportedly more susceptible. The sporoplasm becomes the meront, although the merogony phase is sometimes absent. In some genera, only a few spores are produced from the two phases of division, whereas in others, enormous numbers may result from repeated divisions in either phase. It is rare to find stages other than spores or more than one life stage in infected material. Eventually, infected cells become filled with spores and lyse to release the spores. Generally, if the infected cells are gut epithelial or excretory tubule cells, discharge of spores occurs to the external environment in faeces. Otherwise the spores are not released until after the host dies or is eaten, or the spores are vertically transmitted. Spores liberated into the haemocoel may be encapsulated by haemocytes (phagocytosis) and by subsequent deposition of melanin (Canning 1990). The infection may be localised or generally distributed. Most spores once released from the body can survive for weeks or years if not exposed to sunlight, freezing, drying, or temperatures >35°C (Maddox 1973). The life cycle may be completed in as little as three days. Most microsporidian infections of insects have an incubation period of from five to 10 days before the first mature spores are present (Weiser 1963).

Some infected hosts may appear normal; in others there may be morphological or behavioural changes. For example, moulting abnormalities, reduced or increased size, colour differences (often whitening), reduced feeding, lethargy, and a white faecal exudate may occur although these symptoms are not specific to microsporidian infections. Reduced longevity and fecundity are often the end result of severe infections.

To detect microsporidian spores, impression smears of infected tissue are examined under phase contrast or bright field light microscopy. The smears are stained with Giemsa or haematoxylin to detect both spores and developing stages (Poinar & Thomas 1984). Electron microscopy is necessary for determination of species.

Viruses occur in a wide variety of insects and mites. In many instances the arthropod host vectors the virus to infect a secondary plant or animal host. More than 450 different viruses have been reported from insects and mites. Classification systems are in a state of flux as more and more information is being acquired on biochemical, biophysical, and morphological features; many viruses still await classification. For practical purposes, two main groups are considered: those with inclusion bodies or polyhedra (= occluded viruses), and those without (= non-occluded viruses). Members of the former group are just visible with the light microscope, whereas those of the latter group are only visible with the electron microscope. The virus particles (=virions) in the occluded forms are embedded within a protein matrix which makes the unit more visible. Three such groups are i) the nuclear polyhedrosis viruses (NPVs), 0.5-15μm in diameter, angular in outline, and containing many virions, ii) the granulosis viruses (GVs), 0.15-0.35μ in diameter, oval or ellipsoidal in outline, usually containing one virion, and iii) the cytoplasmic polyhedrosis viruses (CPVs), 0.5-2.5 μ in diameter, variable in shape, and containing several virions. The non-occluded viruses include i) the iridescent viruses (IVs), 130nm-195nm in diameter, ii) the enteroviruses (EVs), 20-30nm in diameter, and iii) the rhabdoviruses (RVs), 70 x 175+nm. The CPVs occur in the host cytoplasm, the rest generally infect the nucleus. NPVs are reported from the following insect orders: Lepidoptera, Hymenoptera, Diptera, and more rarely Orthoptera, Coleoptera, and Trichoptera. GVs are restricted to Lepidoptera. CPVs primarily infect Lepidoptera, but have also been found in Neuroptera, Diptera, and Hymenoptera. IVs have been found in Diptera, Coleoptera, and Lepidoptera. EVs have been reported from vertebrates, insects, and mites. Reed & Desjardin (1978) reported a virus of the EV type in the citrus red mite, *Panonychus citri.* The RVs infect vertebrates, invertebrates, and plants. Viruses observed in the citrus red mite (Smith *et al.* 1959), and the European red mite, *Panonychus ulni*, (Putnam 1970) probably belong in this group.

Most viruses are acquired by the host via the digestive tract. The ingested virus replicates or passes through gut epithelium and enters other susceptible tissues. Transmission is also known to occur transovarially, but there is little information in this regard. Most virus infections produce acute symptoms, but some are inapparent and may manifest themselves only when the host is stressed, such as during overcrowding, starvation, or multiple infection with other pathogens.

The symptoms of infection are quite variable. NPVs induce behavioural changes, loss of appetite, sluggishness, ruptured skin, and kill their hosts. GVs and EVs produce non-specific symptoms that may vary from one insect to another. With CPVs, only the midgut cells are infected which results in loss of appetite and retarded growth. Some non-occluded viruses infect the midgut initially, causing vomiting and diarrhoea, some cause paralysis, but others cause no obvious symptoms in the host.

Occluded viruses are usually detected by examining fresh smears of infected tissue under bright field and phase contrast light microscopy. NPVs and CVs appear refringent (shining white) under both, whereas GVs appear white in bright field and grey in phase contrast. Inclusion bodies dissolve in strong alkali, and do not turn red in the presence of aqueous Sudan III stain (cf. fat droplets). Staining techniques such as the Feulgen-Schiff reaction and Giemsa staining with acid hydrolysis can also be used (see Appendix I). Non-occluded viruses are too small to be seen by these techniques and their presence must be determined by signs in infected hosts and confirmed by electron microscopy. For example, the occurrence of abnormal quantities of birefringent crystals in the body of the citrus red mite is considered diagnostic for the presence of virus (Smith & Cressman 1962, Reed *et al.* 1972).

3 MATERIALS AND METHODS

3.1 *Phytoseiulus persimilis*

Shipments of *P. persimilis* were obtained from three commercial producers, located in Canada, Europe, and the United States. The shipments are designated as from sources A, B, and C, and are not otherwise identified in order to protect commercial interests. Eight separate shipments were received from each source between April and July, 1991. Producers were requested to treat them as they would all normal customer orders. Each shipment consisted of two separate containers of *P. persimilis* on a vermiculite carrier, with the same number of stated contents in each container. Source A containers held 75-120mL of vermiculite in a cardboard tube. All but the first two shipments were packed with gel-type freezer packs to keep the mites cool during shipment. Source B containers held 400-425mL vermiculite per container in plastic bottles, all shipped with freezer packs. Source C contained 400-425mL vermiculite per container in plastic bottles; only shipments 6 and 8 contained freezer packs. The first container in each shipment was used to assess the total number of *P. persimilis* present. The second container was used to assess the percentage of live mites present, and the survival and fecundity of female mites. Specimens were also processed for transmission electron microscopy and for staining to determine if internal pathogens were present. Only two shipments could be evaluated at one time because of the time involved in processing them. Sources A and C were compared, while after the second shipment, Source B was compared with a caged colony of *P. persimilis* established from the first shipment from Source A.

Additional shipments were obtained from Source A, both before and after the reference shipments, and one from California, described as an "Israeli" strain.

Cages containing *P. persimilis* from each of the three sources were maintained and fed on a colony of spider mites originally collected from commercial greenhouses (Plate 3, Fig. 2). Although initially reared on cucumber plants, all subsequent rearing was on green bean plants. Fifteen pots were seeded weekly, three bean seeds per pot. The bean plants were raised in growth chambers (~25°C, 16L 8D) for three weeks to avoid thrips infestations, and then moved to a greenhouse unit. Plants were moved as necessary to another unit for infestation with spider mites. Once well-infested, the plants were placed in predator cages in a third unit. The spider mite population was supplemented daily with addition of infested leaves. After a week, the plants were cut off at the base to ensure movement of mites onto fresh plants.

Number present The total contents of the first container were sieved in small batches through three successive screens (800µ, 350µ, and 88µ sieve opening) under strong water pressure. The first two screens removed carrier material and the final one collected all stages of *P. persimilis*. These were preserved in a 70% alcohol mix and the total number of *P. persimilis* (all motile stages) counted.

Percentage live The contents of each of the second containers were well mixed by rotation of the containers, and approximately 10mL of vermiculite placed in each of five separators. The separator consisted of a small 55mm-diameter petri dish glued to the bottom and centre of a larger 150mm-diameter petri dish containing a solution of water and soil wetting agent to break the surface tension of the water. Two 150 watt incandescent light bulbs were suspended approximately 30cm above the separators. Live mites became very active and in attempting to leave the vermiculite, fell into the soapy water and drowned. After several hours, a lid was placed over the entire separator to prevent evaporation of the solution. After 24h, the water was sieved and mites collected and preserved in alcohol for counting as "live" mites. The vermiculite was similarly sieved to remove "dead" mites. The percentage of live mites in each of the five samples was then calculated. "Live" mites were then cleared in polyvinyl alcohol and the percentage of females with eggs was recorded.

Longevity and fecundity Twenty live female mites were randomly collected from each second container and confined individually on leaf disc arenas. The leaf disc arenas (Plate 3, Fig. 3) were comprised of plastic Millipore® dishes 54mm in diameter. A 30mm-diameter hole was cut in the lid of each dish and covered with screening to allow air movement but prevent escape of mites. The base of the dish was lined with an absorbent material. Initially cotton batten was used for this purpose but it was time-consuming to install and sometimes the mites were trapped and drowned. Later, black landscape felt (Filterbond®) was used. This material could be re-used after soaking in 10% bleach, then rinsing and autoclaving for 30 minutes. Two such felt discs wetted with distilled water were used in each dish. A 30mm-diameter leaf disc cut from long

English cucumber leaves was placed on the felt, upper surface down. Initially, to provide food, 50 spider mites from colonies maintained on bean and cucumber plants were transferred to each disc the day before a shipment was due to provide food. This procedure was very time-consuming so bean leaf discs containing high populations of spider mites were used instead after the second shipments. An advantage of this method was that the discs could be set up at the same time the *P. persimilis* arrived to provide a range of prey stages. Leaf discs were replaced as necessary to provide a surplus of food on good quality plant material. The twenty arenas from each shipment were placed screened-side down in another plastic container lined on the bottom with wet cotton fabric. A broad-opening plastic mesh separated the dishes from the fabric and allowed the exchange of air and water vapour. The large containers were closed to provide high humidity and kept in a growth chamber at 25°C and a 16L:8D cycle. Each arena was examined (except one or two days on weekends) over a seven day period. Records were kept of the number of eggs laid by each *P. persimilis* and whether the mite was live or dead. A mite found dead on day 6 was recorded as living for 5 days, whether or not additional eggs were found. The same rule held where days were missed on weekends, so lifespan sometimes erred on the plus side. Notes were made on any abnormalities in appearance or behaviour. Dead *P. persimilis* were removed and sent for electron microscopy or smeared on glass slides to determine cause of death.

Eggs were removed after counting and placed in Millipore® dishes lined with two layers of moist filter paper. The paper was marked in seven quadrants, one for each day, so that age of eggs could be tracked. The percentage of eggs that hatched was recorded. Mites not laying eggs were examined at the end of the assessment period or on death to determine if they were male or female.

Occasionally, mites could not be found and were presumed to have escaped. All data were discarded for these mites and for males. At the end of a seven day period, the average longevity and fecundity of female mites was calculated. Fecundity was expressed as mean eggs/day/mite based on the number of females in the batch at start-up. Data were also calculated as cumulative number of eggs laid/day by the batch so that changes over time could be assessed. Where a day was missed, the total number of eggs laid was averaged over the two days.

Disease incidence Because the diseases that might be encountered in *P. persimilis* were unknown, the initial batches were subjected to a variety of fixatives, preparation techniques, and histochemical stains. Twenty five to 50 mites in each batch (except Source A, batches 3 and 7), were fixed in Universal fixative (1% formaldehyde, 1.5% gluteraldehyde in 0.12M cacodylate buffer, pH 7.2) and sent to Electron Microscopy Services section at AEC. They were stored in the refrigerator until processed for transmission electron microscopy. Detailed preparation for transmission electron microscopy is described in Appendix II. Later samples were submitted separately based on abnormal body colouration, such as extensive white areas in the gut and Malpighian tubules, or black dots in the abdomen (Plate 1, Figs. 2-6). The normal colouration is orange without markings. The abnormalities showed up best against a red background. Mostly adult female mites were processed, but two series of stains were run on eggs and nymphal stages, one from Source A and the other from Source B.

The staining procedures used (see Appendix I for details) were A: Giemsa stain following acid hydrolysis (to differentiate rickettsia from granulosis virus), B: Giemsa stain following methanol fixation (to stain microsporidian spores), C: slow staining with Giemsa following acid hydrolysis (to stain virus rods and inclusion bodies), D: Giemsa stain and acid hydrolysis without methanol fixation (to differentiate fat globules from crystals), E: Macchiavello stain (to differentiate rickettsia, crystals and spheroidocytes from bacteria and albuminoid crystals), F: Guegin's stain (to differentiate virus from host fat cells and fungi), G: Gram stain (to differentiate Gram-positive bacteria from Gram-negative bacteria), H: Papanicolaou stain (to differentiate nuclei from other cellular components), I: Ziehl-Nielsen stain (to differentiate acid fast from non-acid fast bacteria), J: periodic acid Schiff stain (to demonstrate presence of mucous proteins), K: neutral red stain (to demonstrate the presence of rickettsia, L: lacto-aceto-orcein stain (to show nuclei), M: Masson trichome stain (to differentiate various host tissues), and N: India ink (to demonstrate and to measure microsporidian spores).

Several smear preparation techniques and fixation methods were tried on individual mites. A thin, even film was desired which would remain on the slide during subsequent processing and permit appropriate uptake of stain. Smears were tried in either distilled water, soapy water, 0.1M hydrochloric acid (HCl), or insect Ringer. Fixation methods that were used included heat, flame, Carnoy's solution, methanol, Bouin Dubosq Brasil solution, formaldehyde, Cytoprep®, or 3-aminopropyltriethoxysilane (APTS, albumin treated). Various combinations of stains and fixatives were investigated for batches 1-5, after which mites were separated into those with normal and those with abnormal colouration. Smears of up to eight individuals in each category were prepared, allowed to dry, and stored for staining at a later date. All but one smear of each set was then stained with Giemsa following methanol fixation (Stain B, method 2); the last one was fixed with Cytoprep® and stained by Macchiavello's method (Stain E). The various stains used are listed in Table 8.

3.2 *Amblyseius cucumeris*

Shipments of *A. cucumeris* could only be obtained from suppliers A and C. Four separate shipments were received from each supplier during February and March 1991. The second shipment from source A did not arrive and was replaced with a later one. Each shipment consisted of two containers of *A. cucumeris* with bran mites on bran supplied as food. One container was used to assess the total number of *A. cucumeris* present. The other container was treated similarly to those containing *P. persimilis*, in order to assess longevity, fecundity, percentage live, and percentage of females with eggs.

Number present With 10,000 mites supposedly present in each container, a subsample was used to assess total numbers. The contents of the container were emptied into a dish and mixed well before sampling. Ten mL of the bran mix were collected in a small plastic cylinder which was tapped lightly to settle the bran. The material was then sieved using a strong water spray and the mites preserved as previously described for *P. persimilis*. There were five replicates per container. The total volume of bran in the container was measured in a graduated cylinder in order to extrapolate the sample count to an estimate of total number of mites present.

Percentage live The percentage of *A. cucumeris* live could not be assessed by placing under lights because the mites were less active than *P. persimilis* and were reluctant to leave the curls of the bran. We attempted to determine this information from the preserved material; however, it was difficult to separate dead mites from cast skins of these and bran mites. Besides, the percentage of dead mites appeared to be very low, so we did not make this assessment. The percentage of adult mites with eggs and percentage of adults in the batch were calculated.

Longevity and fecundity The procedures followed were very similar to those described for *P. persimilis*. Initially, 20 adult mites were removed from each batch and placed singly in the leaf disc arenas. Because of concern that bran mites might be carriers of diseases that would affect *A. cucumeris*, they were substituted with first instar western flower thrips larvae for food. Because of the speed of development of the thrips, it was necessary to introduce new thrips larvae almost daily and to remove second stage larvae as a precaution in case they ate predator eggs or damaged predators by defensive thrashing of the abdomen. We did not actually see any evidence of this. Unlike *P. persimilis*, there was a high disappearance rate of *A. cucumeris* from the arenas. This was attributed to the tendency of *A. cucumeris* to leave the leaf arena and conceal itself between the layers of felting, where it was very difficult to find. If the mite had not been seen for two days and no eggs had been laid, it was presumed for recording purposes to have died on the last day no eggs were laid. If detached screening indicated possible escape, all records for that individual were discarded. Twenty five mites were used after the first batch to compensate for some of the losses. Eggs were often laid off the leaf disc, so more care had to exercised in checking arenas than was the case with *P. persimilis*.

Disease incidence *Amblyseius cucumeris* and bran mites were removed from the carrier bran by washing the bran through a series of screens. Samples of mites were fixed in Karnovsky's or Universal fixative for electron microscopy. Ten predator mites and ten bran mites were smeared individually in a small drop of distilled water on glass slides and allowed to dry for later staining for pathogens. Nine smears of each batch were stained with Giemsa (Stain B, method 2), and one with Macchiavello's stain (Stain E).

3.3 *Encarsia formosa*

Shipments of *E. formosa* were obtained from Sources A and C. A total of four shipments from each source were received in February and March 1992. Two thousand *E. formosa* were ordered from each source. Source A shipment consisted of 20 cards bearing parasitized whitefly scales (100 wasps/card expected), Source C of 42 cards (50 wasps/card expected, with two extra cards).

Number present and percentage live On arrival, 20 cards from each source were checked under the microscope for holes in the black, parasitized scales indicating pre-emergence of *E. formosa* (i.e. emergence prior to receipt). Cards were then placed individually in small, snap-top plastic jars and maintained in a growth chamber at a constant temperature of 25°C and 16L:8D photoperiod. After seven days, cards were transferred to another set of jars and *E. formosa* that had emerged in the first jar were preserved in alcohol for counting. The cards were held for another seven days and a record kept of any further emergence. To determine the percentage emergence, a count was made of total number of black pupae per card and those pupae with emergence holes. To do this, a drop of water was placed on the pupae to soften the glue, and pupae were removed one at a time with fine forceps and categorized. Failure to do this can result in missing those with emergence holes on their side because they are difficult to see. Pupae with visible exit holes were removed and counted first. Data for exit holes were checked against the number of emerged wasps plus the pre-emergence count. Results are reported as the total percentage of parasitized scales where emergence occurred, the percentage of scales where pre-emergence occurred, and the mean number of emerged wasps per card.

Longevity and fecundity Due to limited resources it was not possible to determine these parameters. The procedures are more complicated for a parasite than a predator because of feeding requirements and internal deposition of eggs within the host.

Disease incidence Approximately 40 adult female *Encarsia formosa* were removed from the jars in which they had emerged. They were placed in fixative (Karnovsky's and/or Universal), and submitted for transmission electron microscopy. Another ten *E. formosa* from each batch were placed individually on glass slides in a small drop of distilled water and smeared. They were air dried and stained at a later date. Nine smears of each batch were stained with Giemsa (Stain B, method 2), and one with Macchiavello's stain (Stain E).

4 RESULTS AND DISCUSSION

4.1 *Phytoseiulus persimilis*

Number present (Table 3) The mean number of *P. persimilis* present in the container varied for each source and for different batches (stated contents indicated in parentheses): 326 to 8461 for Source A (1000), 1774 to 4345 for Source B (2000), and 1254 to 3579 for Source C (2000). Two shipments from both sources A and C were seriously short on mites present. Source A shipments showed the most variation in numbers between batches.

Percentage live (Table 3) The percentage of live mites was high in shipments from source B (83.4 to 98.0%), but was sometimes low in those from Sources A (29.5 to 87.4%) and C (40.6 to 88.8%). Although stated contents may still be present, a high percentage of dead mites suggests that the survivors may not be very fit. The percentage of females arriving with eggs was very low. Sample means were zero except Source A, batch 4 (3.2%) and batch 8 (0.3%), and Source C, batch 1 (0.2%).

Longevity and fecundity (Tables 3 and 4) Mite survival from those in the established AEC colony ranged from an average 5.50 to 6.25 days over a seven day experimental period. Comparable figures for batches from Sources A, B, and C were 1.35 to 6.53, 2.37 to 5.35, and 2.42 to 5.54 days respectively. The percentage of live mites after five days ranged from 5.0 to 94.7% for Source A, 15.0 to 65.0% for Source B, 10.5 to 64.3% for Source C, and 73.7 to 90.0% for AEC mites. The figures for the commercial sources generally fell far short of the 80% suggested by IOBC guidelines (van Lenteren & Steinberg 1991).

Egg laying also differed from that of the established AEC colony. AEC mites laid an average of from 2.35 to 3.92 eggs/female/day, which is within the range of 2.4 to 4.0 reported in the literature (summarized in Table 1). Female mites from Sources A, B, and C laid 0.09 to 2.71, 0.19 to 1.97, and 0 to 1.20 eggs/day respectively, considerably lower than the average. A high percentage of these eggs hatched. After the first day, the number of eggs laid per day by surviving mites in the arenas remained nearly constant; the death of the others lowered the overall production for the batch.

Colonies were set up in cages with mites from Sources B and C, but the mites did not survive long.

Disease incidence **Light microscopy** A total of 720 slides was processed (Table 8). The best smears were obtained by crushing fresh mites in a very small drop of distilled water on a glass slide. Insect Ringer solution crystallised and obscured details. The most satisfactory preparations were obtained with methanol fixation followed by Giemsa staining (Stain B, method 2). Macchiavello staining showed better clarity when the mites were smeared with water. The method of fixation did not affect the result. Several types of microorganisms were found in the smeared material (Table 11, Plates 4-10). Early assessments of preparation methods for degree of differentiation from background material were coloured by an artifact (Plate 10, Figs. 3-6) believed to be a bacterium, and so described by Arutunjan (1985). We later determined it to be a calcium salt; its presence produced the "white gut" evident in many specimens.

Different *P. persimilis* individuals, both within batches and among suppliers, contained different amounts and types of microorganisms, making it very difficult to compare preparation methods. Apart from "white gut", it was not possible to relate other colour differences in the mites such as mottling or black dots to specific microorganisms. Reasons for this may be that multiple infections were the rule rather than the exception, or apparently healthy mites would also be infected, or the symptoms may have been virus-related and could not be detected by light microscopy. Emaciated, translucent mites did not survive. Microorganisms which were clearly **bacteria** (Plate 9, Figs. 1, 2) were only found in dead and moribund mites, and are suspected of being secondary invaders rather than primary pathogens. Almost all mites were infested with coccoidal microorganisms of various sizes (Plates 6, 7). Most cocci were blue or violet, though some were red. We were not able to determine if these were **rickettsial, viral**, or **bacterial**, but we suspect most were rickettsia. Colour differences can occur using the same stain. Sometimes red crystalline stages of **rickettsia** were clearly present (Plate 7, Fig. 5), particularly in egg and early nymphal stages of the mite. **Fungi** and fungal spores were found only in small amounts (Plate 8, Figs. 1-4) and fungal infections were rarely evident. **Microsporidia** (Plates 4, 5) were easiest to identify in the spore stage. They were found in several individuals in six of 10 shipments from Source A, and were also common in earlier samples from this source. Spores were found in a few individuals in batches 3, 6, and 8 from Source B, and batches 5 and 8 from

Source C. The "Israeli" strain mites had a high incidence of infection of both microsporidia and rickettsia, and may have contributed to contamination of colonies to which they were added. Based on differences in spore size and presence or absence of sporophorous vesicles, more than one microsporidia species is believed to be present, although some species do have two spore types. Merogonial and early sporogonial stages were rarely found in adult female mites, but were found instead in egg and early nymphal stages (Plate 5, Figs. 1-6). No occluded **virus** was identified, although occasionally crystals similar to those reported from citrus red mite infected with virus were noted (Plate 8, Fig. 6). One batch of spider mites that served as food for *P. persimilis* was also checked. They appeared to be mostly free of microorganisms. One incidence of small coccoid forms was found (Plate 16, Fig. 6).

Electron microscopy Because of the time involvement and complexity of the process, very few mites could be processed by this method (59 in total). Several blocks were prepared but only 1-7 mites in each batch were actually cut by transverse section through the abdomen and examined by transmission electron microscopy. There is only very basic information published (in Russian) on the internal anatomy of *P. persimilis*, (see Plate 17, Fig. 1 for a stylized presentation of the internal anatomy) and recognition of artifacts and tissues in electron micrographs is a skill still being acquired. Microorganisms found in *P. persimilis* are tentatively listed in Table 11. Micrographs were submitted to several invertebrate pathologists (J.I.R. Larsson, University of Lund, Sweden; E. Beerling, University of Amsterdam, The Netherlands; J.J. Bectel, USDA, Gainsville, Florida, USA; J. Weiser, WHO Collab. Centre Vector Pathol., Czech Republic; J.V. Maddox, Illinois Natural History Survey, USA; G. Šuťáková, Slovak Academy of Sciences, Slovakia; M. Goettel, CDA, Lethbridge, Alberta; B.A. Keddie, University of Alberta, Edmonton, Alberta). The presence of **microsporidia** and **rickettsia** was confirmed, but individuals were uncertain about the identity of other microorganisms. Microsporidian spores were readily identifiable (Plate 18, Figs. 1-5). Four spore types were found in Source A and "Israeli" strain material, none in Source B or C. The first type (rare) (Fig. 1) measured ~1.11 x 4.28μ with 3-4 polar filament coils; the second (Fig. 2) measured ~1.19 x 2.64μ with 9-10 coils; the third (Fig. 4) measured ~1.12 x 2.21μ with 3-4 coils, and the fourth measured ~0.91 x 1.98μ with 6-8 coils. Another spore-like object (Fig. 3) measuring ~1.14 x 3.55μ had no visible polar filament coils but may be an undeveloped spore.

Microsporidian spores were found in the cytoplasm (Plate 19, Figs. 1 and 2) and also in the egg within a gravid female (Plate 20, Fig. 1). They were not found in the digestive cavity. Merogonial stages were not observed. Rarely, sporophorous vesicles containing developing spores were found (Plate 18, Fig. 6). Melanized material was evident in sections from all sources (Plate 19, Fig. 3) and may be spores that have been killed by host reaction, or possibly by antibiotics.

The calcium crystals noted during light microscopy were found mostly within the Malpighian tubules, but also in the digestive tract, cytoplasm, and legs. They appear to be composed of layers of material in a dumbbell shape (Plate 20, Figs. 3, 5, and 6).

Rickettsia-like organisms (Plate 21, Figs. 1-6, and Plate 22, Figs. 1, 3, and 4) sometimes filled the whole body of the mite, making it difficult to understand how it still functioned. Various life stages were present, particularly those forms termed bacterial and dense rickettsia (Šuťáková 1988). Bacterial rickettsia averaged 0.4 x 1.4μ and dense rickettsia 0.3 x 0.8μ. The bacterial rickettsia were quite long but similar in size to Type B organisms reported from the mite *Metaseiulus occidentalis* (Hess & Hoy 1982). The length being measured may be reflecting that just prior to division. Rickettsia were also found in one spider mite of several checked, but did not appear to be common in this host. Melanized objects were also noted in association with rickettsia (Plate 22, Fig. 4).

A non-occluded, rod-like **virus** was located within the developing egg of gravid female mites (Plate 23, Figs. 2 and 3). It is very small (<50nm wide) and similar in size to enteroviruses. This type of virus would not be visible using light microscopy.

4.2 *Amblyseius cucumeris*

Number present (Table 5) The stated contents in each container were 10,000 *A. cucumeris*. The mean number far exceeded stated contents in shipments from Source A (17,090 to 34,392 mites), whereas shipments from Source C were less than stated (3,460 to 8,766 mites). In neither case were the numbers consistent between batches. The percentage of adults containing eggs on arrival ranged from 38.8 to 62.0% for Source A and 17.8 to 58.3% for Source C. This is greater than mites in *P. persimilis* shipments and may be a consequence of *A. cucumeris* being shipped with food (bran mites). The percentage of adult mites was lower in Source A (28.1 to 36.5%) batches than in those from C (43.2 to 54.6%).

Survival and fecundity (Tables 5 and 6) Mean mite survival over a seven day experimental period ranged from 2.42 to 5.42 days for Source A mites and 3.55 to 4.61 days for Source C. *Amblyseius cucumeris* was more difficult to raise than *P. persimilis* because of the almost daily need to reintroduce thrips at the first larval stage as food, and because quite often the whole container had to be searched to find the mite.

Source A mites laid averages of 0.40 to 1.05 eggs/day, and Source C mites 0.29 to 0.58 eggs/day. These figures are lower than the 1.5 to 1.6 eggs/day reported in the literature (Table 2).

Disease incidence **Light microscopy** A total of 158 slides of *A. cucumeris* were processed (Table 9), and 70 of *T. putrescentiae*. A wide range of microorganisms was found, including **microsporidia**, **rickettsia**, **bacteria**, **fungi**, **yeast**, and **gregarines** (Tables 12, 13 , Plates 11-14). Two quite distinct types of **microsporidia** were found, one with a round spore, found in *T. putrescentiae* from both Sources A and C (Plate 13, Figs. 1 and 2), and the other oval, appearing stirrup-shaped, found in *A. cucumeris* from Source A and in *T. putrescentiae* from both sources (Plate 11, Fig. 1, and Plate 13, Fig. 3). The oval spore closely resembles *Nosema steinhausi*, described by Weiser (1956) as infesting *T. noxius*. Coccoid forms and short rods were quite common (Plate 11, Figs. 3 and 4, Plate 12, Figs. 1-5, Plate 13, Figs. 4 and 5). Some of these we believe to be **rickettsia**. In a few mites from both sources, cells infected with short, rod-like forms were noted. "White gut" had also been evident to a lesser extent in *A. cucumeris*, and a few crystals similar to those found in *P. persimilis* were noted. Birefringent crystals of a more rectangular shape were sometimes found (Plate 11, Fig. 5). Two stages or types of **gregarines** (Plate 14, Figs. 1-4) were found in *T. putrescentiae*, the most common being a septate eugregarine (Plate 14, Fig. 4). It was also observed occasionally in smears of *A. cucumeris*. There are six records of gregarine infections in mites (Lipa 1982), but none that fits the description of those found in this study.

Electron microscopy Processing was completed on 29 mites. Microorganisms found in *A. cucumeris* and *T. putrescentiae* are listed in Tables 12 and 13 respectively. **Microsporidian** spores and sporophorous vesicles were found in bran mites from Source C (Plate 19, Figs. 4-6). The spores were of the round type with 2-3 polar filament wraps, and measured on average

1.3 x 1.5µ. They are of the *Chyrtridiopsis*-type and may represent a new genus (R. Larsson, personal communication). In one mite they were present in the same tissues as large numbers of **rickettsia** (Plate 19, Fig. 4). Both spores and rickettsia were released into the gut and expelled through the anus (Plate 20, Fig. 2). The oval spores noted in light microscopy were not found. Non-occluded **virus** similar to that found in *P. persimilis* was present in eggs within gravid females of both *A. cucumeris* from Sources A and C (Plate 23, Figs. 1 and 5), and in *T. putrescentiae* from Source A (Fig. 4).

4.3 *Encarsia formosa*

Number present and percentage live Nearly all wasps emerged from parasitized pupae on the card during the first seven days (Table 7). The percentage of wasps that emerged prior to receipt was low except for those in the second shipment from Source A and the fourth from Source C. It was evident from the results that two types of cards were present in this particular Source A shipment. It was determined that one half of the batch (b) had been repackaged and was originally from Source B. The total percentage emergence (a measure of percentage live) ranged from 38.3 to 59.05% for Source A, and from 43.38 to 65.78% for Source C. This is much lower than the >90% emergence reported by one commercial producer in tests carried out just prior to shipment, and the 80% stated as an acceptable emergence level (Ravensberg 1991). Increasing the humidity in the jars by screening and enclosing in high-humidity chambers did not increase the percentage emergence. The total number of emerged wasps per card ranged from 52.85 to 107.3 for Source A (100 expected) and 34.75 to 72.3 for Source C (50 expected).

Disease incidence **Light microscopy** A total of 60 slides were processed (Table 10). Very few types of microorganisms were found (Table 14). Most common were coccoid forms of varying sizes (Plate 15, Figs. 1-3) and irregular objects (Figs. 4 and 5). No microsporidia spores were found. Cells with the cytoplasm infected with short, rod-like forms were present in individuals from both sources (Plate 16, Figs. 1-3). They are similar to those described by Cowdry (1923) as **rickettsia**. The nucleus in some cells also is granular in appearance and possibly also infected (Plate 16, Fig. 1). Crystals similar to those found in virus-infected citrus red mites were occasionally present (Plate 16, Figs. 4 and 5).

Electron microscopy Thirteen individuals were processed. Unidentified crystalline artifacts were found in a few individuals (Plate 22, Fig. 5), possible rickettsia in one from Source A, and non-occluded virus particles in another from Source A (Plate 23, Fig. 6).

5 CONCLUSIONS

The results clearly demonstrate that Alberta growers were justified in questioning the quality of the biologicals that they were purchasing. Shipments of all three biologicals were in many cases short on stated contents. Although in some cases they were in excess of stated contents, the enormous variation in numbers present makes it impossible to accurately assess the "effective dose", for present or future reference. The "quantity" aspect is bad enough, but the quality of those individuals that were present is also of concern. The mites performed poorly in a favourable environment with food provided. Conditions in a commercial greenhouse are not so kind.

Phytoseiulus persimilis were clearly starved on receipt, as few females carried eggs and most were emaciated in appearance. "White gut" was very common in mites from all sources. Although we could not relate it directly to a particular disease, the excessive presence of calcium crystals suggests a physiological imbalance or waste accumulation that may be an indirect effect of one or several disease agents. The AEC colony was derived from a shipment that performed well on arrival, but it too deteriorated over time. At one time *P. persimilis* were shipped on leaves with food provided; many growers still prefer to receive them this way despite the inconvenience of distribution, because they arrive in better condition.

Amblyseius cucumeris were shipped with food and most females carried eggs. They appeared more lively than *P. persimilis* on receipt but did not perform that well in the arenas. The change in diet from bran mites to thrips may have had a negative effect on survival in the arenas but the fourth batch from Source A performed reasonably well in this system. Pre-emergence of *E. formosa* indicates improper storage. These wasps are usually destroyed when setting out cards or perform poorly because they have spent time confined. They may not have sufficient energy to fly to a new food source after release. The flying and egg-laying capacity of the wasps were not assessed during these trials but IOBC guidelines are currently being developed for this assessment. The low percentage emergence raises a question about the viability of the survivors.

Most shipments were received from source within 48h of shipping and processed immediately. Occasionally shipments arrived late in the day. These were refrigerated overnight and processed early the following day. The sources of the quantity and quality problems are open to question. Quantity problems relate directly back to the supplier, who should be running checks on each batch. Athough it is not realistic to expect the exact numbers specified on the container, a minimum number should be present. Excessive variation in numbers seen in this trial preclude adequate assessment of the impact of a predator or parasite. Quality problems are more difficult to pinpoint. Deterioration from prolonged storage, improper packaging that does not keep the product cool during the shipping period, and inadequate food during storage/shipping are factors also under the direct or indirect control of the producer. These are not the responsibility of the end purchaser. A packaging and expiry date on each package would assist consumers in evaluating whether the product as received is likely to perform. Starved biologicals may not recover no matter how well they are treated on arrival, so a food supply in transit should be provided. The time from harvesting to receipt by end user is critical. Prolonged storage sometimes occurs and leads to deterioration in performance. This is compounded when repackaging by distributors occurs. Consumers need to be wary if the time from harvesting to delivery is longer than 48 hours. More research on the effects of storage needs to be conducted and the results published.

Other possibilities for poor performance are genetic inbreeding and disease. Most present stocks of *P. persimilis* originate from the Chilean material with a limited genetic pool. Field collections of populations from the same source do not really change this pool. They also introduce an unknown element as far as disease is concerned. As far as I am aware, there has been no recent attempt, documented or otherwise, to go back to the native populations to add to the pool.

Producers often assert that the biologicals do well in the rearing environment, but this performance may be different when subjected to the extremes of most greenhouse and field environments. Although biologicals cannot be expected to perform under extreme conditions, they should be able to do so under a normal range of fluctuating conditions. Some idea of "normal" should be stated on the container. As timing of releases and early establishment is usually necessary for good control, it is not acceptable to receive far fewer viable organisms than ordered.

Some suppliers/producers rely on complaints from consumers to alert them to problems in production. Very few consumers, however, are in a position to be able to do more than make a very general assessment of the condition of biologicals on receipt. In practice, the response has often been to replace the shipment or blame the shipper or grower rather than to seriously examine the reasons for shortcomings in field performance.

Like most living organisms, the biologicals under discussion are subject to diseases. These have been poorly documented, and generally appear to have been handled by attempting to decrease stress on that culture, or by supplementing or replacing it with one from another supplier with no knowledge of the condition of that culture either. There are only two documented cases of microorganisms in *P. persimilis* (Šuťáková & Rüttgen 1978, Šuťáková 1988), one in *A. cucumeris* (Beerling & van der Geest 1991), and two in *E. formosa* (Stouthamer *et al.* 1990, 1993, Zchori-Fein *et al.* 1992), in the latter case dealing with the influence of rickettsia in determining the female-dominated sex ratio. The smaller producers are often not aware of the potential of disease, and the larger producers are reluctant to discuss the problem as they are afraid of undermining consumer confidence and sales in a competitive market. Nevertheless, consumer confidence is presently being more seriously undermined by poor quality and performance. We found several microorganisms infecting all three biologicals. These included viruses, microsporidia and other protozoa, rickettsia, fungi and bacteria. Specific identification, epidemiology, and pathogenicity await further study. Light microscopy, Giemsa, and Macchiavello stains are useful in detecting the presence of some types of microorganisms, but cannot determine species or tissues involved. Thin sectioning, centrifugation, and other techniques are required to gain more information on specific organisms, and electron microscopy to examine the fine detail needed for species identification and to detect non-occluded virus. This is a long and complex process, particularly in light of the lack of information on internal anatomy of the host. Further identification of many of the present samples awaits expertise that we have not yet been able to locate.

The poor performance of the predator mites in many batches and poor recovery after receipt points to a definite health problem.

Microsporidia are known to reduce longevity and fecundity. In *P. persimilis*, the mode of transmission appears to be transovarial. In *A. cucumeris*, one species was found in the rearing host also, increasing the likelihood of cross-contamination. This species has not yet been detected through electron microscopy, so the tissues in which it is found are not yet known and the mode of transmission cannot be speculated on. Round spores of another species were found in the intestine of the bran mite and were discharged through the anus, so horizontal transmission is more likely. This would fit better into the life style of the bran mite, which is found in concentrations.

Rickettsia may be pathogenic or non-pathogenic. Those that are non-pathogenic may alter the sex ratio of the host in unacceptable ways or result in mating incompatibilities. Rickettsia of this type (*Wolbachia*) were confirmed by DNA analysis (Scott O'Neill, Yale University School of Medicine) in *P. persimilis* and *T. putrescentiae.* The sex ratio of the predator, parasite and rearing host needs to be monitored closely in the rearing facility. Antibiotics can remove rickettsias, but there may be unforseen consequences. The rickettsia may be symbiotic and necessary to the health of the host, or crossing of "clean" with infected individuals may result in no progeny. This has practical implications where growers bring in supplies from more than one company, or rearing facilities add new stock to their cultures.

The impact of the non-occluded virus that was found in developing eggs of *P. persimilis*, *A. cucumeris*, and *T. putrescentiae* is not known.

All of the microorganisms found may be of limited significance in a well-maintained culture under ideal conditions, but may have a profound impact in combination, or when the host is stressed during shipping or in its intended final destination in field or greenhouse. That there are problems with health and performance of commonly used biological control agents has clearly been shown by this study, and is backed up by many consumer complaints.

The Federal Government in Canada, the United States, and some European countries are moving to regulate biological controls. The possibility of such a damper being placed on a fledgling industry at a time when environmentally friendly alternatives to chemical pesticides are urgently needed is providing a strong incentive to the biological control industry to develop its own guidelines. Quality control should benefit substantially from this process. Governments should assist by providing support for studies in invertebrate pathology. There is currently no study on risks associated with bringing in unwanted pathogens with biological control agents.

6 IMPLICATIONS FOR ALBERTA'S AGRICULTURAL INDUSTRY

The use of biological control agents within integrated pest management programs has many possibilities in Alberta, both in protected crops like greenhouses and in weed control in field crops and pastures. Research efforts have generally been of a cooperative nature, partly because this makes good scientific and economic sense, but also because pests have few inhibitions about Provincial or national borders, and nor for that matter do the biological control agents. Most biological control agents are of foreign origin and will need to clear Federal regulatory agencies before being accepted for use. Studies designed to evaluate the effectiveness of an agent have occasionally attempted to monitor actual release rates, but have not considered whether individuals are healthy or not. This may lead to rejection of a potentially good predator or parasite on false assumptions. The consumer may receive a sick batch of biologicals, be disappointed in their performance, and reject biological control as an option.

It is beyond the capability of the consumer to do more than make a cursory check to see if there are an unusual number of dead or missing biologicals in a batch. Commercial rearing facilities range from sophisticated international operations to basement entrepreneurs. Quality control has only recently become a major issue, and this has been partly a response to consumer demands and market competition, but also a response to the spectre of future government regulation. Some regulation is certainly necessary, but if the fledgling industry is to be free to experiment and compete with the pesticide industry, it should not be unduly onerous. The research conducted under this Farming for the Future project has fostered an awareness of the need to learn more about the impact of microbiological organisms affecting biological control agents. A diagnostic facility for invertebrate pathogens is required as a first step, and research on the identity and impact of the major pathogens should be encouraged.

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Table 1. Reported life histories of *Phytoseiulus persimilis*

Investigator	Stock Origin	Temperature Range	Development Time (days)	Pre-oviposition Period (days)	Eggs/ Day	Longevity (females) (days)	Sex Ratio (F:M)
DOSSE (1958)	CHILE	50-77°F	11.9 (4.6-12.2)	-	-	-	1.6:1
RAGUSA (1965)	SICILY	77-82°F	5-12	-	4	22-25	-
LAING (1968)	CHILE	58.5-83°F	7.4	3	2.4 (0-6)	29.6 (12-50)	4.1:1
KENNETT & CALTAGIRONE (1968)	CHILE SICILY	72-80°F "	5 5	-	3.47 3.72	-	- 3.75:1
HESSEIN (1976)	LIBYA?	25°C (77°F)	5 (4-6.5)	3 (2-4)	-	-	2.8:1
AMANO & CHANT (1977)	CHILE	23°C (73°F)	6.13	1.34	2.91	47-53	-

Table 2. Reported life histories of *Amblyseius cucumeris*

Investigator	Stock Origin	Temperature Range	Development Time (days)	Pre-oviposition Period (days)	Eggs/ Day	Longevity (females) (days)	Sex Ratio (F:M)
EL-BADRY & ZAHER (1961)	EGYPT	85°F (29°C)	3.5 (3-4.5)	2.6 (2-5)	1.6 (1-3)	40	-
KOLDOCHKA (1985)	?	26°C	6.29	-	-	-	-
GILLESPIE & RANEY (1985)	HOLLAND	20°C	11.1	3.2+	1.5 (0-6)	-	-
		25°C	8.7	-	-	-	-
		30°C	6.3	-	-	-	-

Table 3. Performance of *Phytoseiulus persimilis* from three commercial sources, 1991

Source	Batch	Date Received 1991	Stated Contents	Actual Contents	% ± SD Live	Mean Longevity ± SD (days)	Mean ± SD Eggs/Day	Mean % Egg Hatch	Means % Survival After 5 Days
A	1	10 Apr	1000	8461	87.4 ± 4.7	6.53 ± 1.22	2.71 ± 1.37	98.3	94.7
	2	24 Apr	1000	3134	52.6 ± 0.9	5.58 ± 2.27	2.61 ± 1.44	98.0	73.7
	3	8 May	1000	4028	29.5 ± 5.9	1.83 ± 1.79	0.11 ± 0.28	85.7	11.1
	4	23 May	1000	632	77.2 ± 8.2	5.74 ± 1.33	2.09 ± 1.18	98.9	78.9
	5	7 June	1000	5009	70.4 ± 6.2	2.10 ± 1.68	0.31 ± 0.72	97.9	15.0
	6	26 June	1000	888	72.0 ± 10.1	2.15 ± 1.98	0.12 ± 0.43	100	10.0
	7	5 July	1000	326	86.8 ± 2.8	3.50 ± 2.01	0.38 ± 0.56	98.1	30.0
	8	17 July	1000	4046	39.6 ± 13.0	1.35 ± 1.90	0.09 ± 0.42	100	5.0
C	1	10 Apr	2000	3465	86.4 ± 9.4	5.54 ± 1.45	0.71 ± 0.46	100	64.3
	2	24 Apr	2000	-[1]	88.0 ± 4.6	4.47 ± 1.42	0.45 ± 0.41	100	47.1
	3	8 May	2000	3579	88.8 ± 8.9	2.89 ± 1.76	0.43 ± 0.42	80.1	10.5
	4	23 May	2000	1421	60.8 ± 10.9	3.75 ± 2.36	0.79 ± 1.23	98.2	40.0
	5	7 June	2000	2321	80.3 ± 10.4	2.84 ± 1.54	0.16 ± 0.25	90.5	21.1
	6	21 June	2000	3127	68.0 ± 7.5	2.65 ± 1.73	0.09 ± 0.16	100	15.0
	7	5 July	2000	1254	75.2 ± 15.2	2.42 ± 1.12	0	-	10.5
	8	19 July	2000	1906	40.6 ± 23.0	3.50 ± 2.12	1.20 ± 1.48	98.2	35.0
B	1	17 Apr	2000	1774	83.6 ± 11.6	4.14 ± 2.60	0.50 ± 0.89	100	64.3
	2	24 Apr	2000	4345	91.6 ± 8.3	3.33 ± 2.52	0.97 ± 1.51	100	27.8
	3	2 May	2000	2602	87.4 ± 16.0	3.06 ± 1.48	0.32 ± 0.49	100	25.0
	4	15 May	2000	2545	83.4 ± 7.9	5.35 ± 2.06	1.97 ± 2.02	98.9	65.0
	5	30 May	2000	2584	93.8 ± 3.9	3.10 ± 1.74	0.46 ± 0.67	100	15.0
	6	13 June	2000	3089	91.8 ± 4.0	2.37 ± 2.03	0.19 ± 0.47	100	15.8
	7	26 June	2000	3064	98.0 ± 2.3	3.80 ± 2.26	1.09 ± 1.25	99.3	40.0
	8	11 July	2000	1919	88.4 ± 8.0	3.37 ± 1.98	0.29 ± 0.45	100	31.6
AEC	1	-	N/A	N/A	N/A	-	-	-	-
	2	-				-	-	-	-
	3	2 May				5.65 ± 2.43	3.92 ± 1.89	100	75.0
	4	16 May				6.25 ± 1.37	3.72 ± 1.07	96.9	90.0
	5	30 May				5.71 ± 2.02	2.59 ± 1.20	100	82.4
	6	13 June				5.58 ± 2.14	2.35 ± 1.23	99.4	73.7
	7	-				-	-	-	-
	8	11 July				5.50 ± 2.46	2.41 ± 1.84	96.7	75.0

[1] Sample Lost

Table 4. Cumulative number of eggs laid by *Phytoseiulus persimilis* over 7 days

Source	Batch	Day							n
		1	2	3	4	5	6	7	
A	1	0.68	4.94	-	-	16.31	17.63	18.95	19
	2	0.42	2.47	-	9.05	12.05	15.52	18.26	19
	3	0.11	0.50	-	-	0.78	0.78	0.78	18
	4	0.16	-	6.00	8.26	11.05	13.31	14.63	19
	5	-	1.00	1.40	1.90	2.20	2.20	2.20	20
	6	0.10	0.35	-	-	0.85	0.85	0.85	20
	7	-	-	1.30	1.60	1.95	2.45	2.65	20
	8	0	0.15	-	0.45	0.55	0.65	0.65	20
B	1	0.43	1.57	2.28	-	3.35	3.49	3.49	14
	2	0.61	1.89	-	4.17	4.84	5.90	6.79	18
	3	0.31	-	2.00	2.25	2.25	2.25	2.25	14
	4	0	-	5.45	-	9.85	11.65	13.80	20
	5	0.35	-	-	3.25	3.30	3.30	3.30	20
	6	0	-	0.68	1.21	1.32	1.32	1.32	19
	7	0.60	2.75	-	-	7.45	7.45	7.65	20
	8	0.32	-	1.69	1.80	2.01	2.01	2.01	19

Source	Batch	Day							n
		1	2	3	4	5	6	7	
C	1	1.00	4.62	-	-	5.00	5.00	5.00	13
	2	0.53	2.00	-	3.12	3.18	3.18	3.18	17
	3	1.16	2.84	-	-	3.00	3.00	3.00	19
	4	0.10	-	2.40	3.55	4.45	5.10	5.55	20
	5	-	0.74	0.95	1.00	1.05	1.10	1.10	19
	6	-	0.45	0.60	0.65	0.65	0.65	0.65	20
	7	-	-	0	0	0	0	0	19
	8	-	3.10	4.45	5.65	6.95	7.80	8.40	20
AEC	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	6.20	-	13.3	16.6	19.75	23.50	27.45	20
	4	1.75	-	12.45	-	19.35	22.35	26.05	20
	5	4.29	-	-	14.47	15.94	17.82	18.11	17
	6	0.84	-	6.47	7.79	10.11	13.43	16.43	19
	7	-	-	-	-	-	-	-	-
	8	3.25	-	8.40	9.95	12.05	14.55	16.90	20

Table 5. Performance of *Amblyseius cucumeris* from two commercial sources, 1992

Source	Batch	Date Received 1992	Staged Contents	Actual Contents ± SD	% Adults With Eggs ± SD	% Adults	Mean Longevity (days) ± SD	Mean Eggs/Day ± SD	Mean % Survival After 5 Days
A	1	12 Feb	10,000	29,380 ± 4,453	54.0 ± 2.8	35.6	2.42 ± 2.36	0.40 ± 0.49	15.8
	2	26 Feb	10,000	34,392 ± 8,208	62.0 ± 3.7	35.4	4.57 ± 2.89	0.63 ± 0.68	65.2
	3	4 Mar	10,000	17,090 ± 1,468	38.8 ± 5.7	28.1	4.25 ± 2.15	0.48 ± 0.38	41.7
	4	11 Mar	10,000	19,940 ± 1,898	40.9 ± 5.0	36.5	5.42 ± 2.41	1.05 ± 0.65	70.8
C	1	14 Feb	10,000	3,948 ± 2,170	52.2 ± 13.8	53.5	3.55 ± 3.11	0.58 ± 0.65	45.5
	2	20 Feb	10,000	5,360 ± 772	17.8 ± 6.0	49.3	4.36 ± 2.92	0.40 ± 0.54	59.1
	3	27 Feb	10,000	3,460 ± 489	55.8 ± 10.1	54.6	4.61 ± 2.44	0.29 ± 0.22	47.8
	4	5 Mar	10,000	8,766 ± 1,630	58.3 ± 9.2	43.2	3.56 ± 3.12	0.44 ± 0.54	40.0

Table 6. Cumulative number of eggs laid by *Amblyseius cucumeris* over 7 days.

Source	Batch	Day							n
		1	2	3	4	5	6	7	
A	1	0.74	-	2.11	-	2.64	2.69	2.80	19
	2	1.09	1.83	-	-	3.22	3.96	4.39	23
	3	1.21	2.25	-	-	2.96	3.09	3.38	24
	4	0.71	2.0	-	-	5.92	6.25	7.33	24
C	1	-	1.09	-	2.14	2.82	3.59	4.09	22
	2	-	0.59	1.09	1.64	2.0	2.36	2.77	22
	3	1.35	-	-	1.61	1.74	1.83	2.05	23
	4	0.24	-	-	1.76	1.96	2.56	3.08	25

Table 7. Performance of *Encarsia formosa* from two commercial sources, 1992

Source	Batch	Date Received 1992	% Emerged (14 days) ± SD	% Pre-emerged ± SD	Total % Emergence	Mean Emergence/ Card After Receipt ± SD	Mean Total Emergence/Card *
A	1	12 Feb	44.13 ± 6.37	5.44 ± 4.64	49.51	78.75 ± 12.87	88.45
	2a	26 Feb	56.43 ± 9.79	2.62 ± 2.28	59.05	102.60 ± 25.83	107.30
	2b	26 Feb	27.89 ± 6.69	21.35 ± 4.59	49.45	38.30 ± 12.37	67.40
	3	2 Mar	35.81 ± 18.62	2.54 ± 1.76	38.35	49.35 ± 33.25	52.85
	4	11 Mar	53.15 ± 9.17	1.54 ± 1.14	54.69	84.70 ± 17.39	87.15
C	1	13 Feb	51.79 ± 20.17	4.17 ± 3.48	55.96	44.10 ± 23.15	47.65
	2	20 Feb	57.71 ± 12.79	1.62 ± 1.51	59.33	60.65 ± 20.02	62.35
	3	27 Feb	39.64 ± 18.89	3.74 ± 4.99	43.38	31.75 ± 15.99	34.75
	4	5 Mar	55.82 ± 5.78	9.96 ± 4.01	65.78	61.35 ± 13.74	72.30

*Expected mean = 100 for A and 50 for C. n=20.

Table 8. Stains used for detection of microorganisms in smear preparations of *Phytoseiulus persimilis*

Source	Batch	Date received (1991)	Date processed (1991)	No. of slides	Stain used (see Appendix I)
A	1	10/4	10/4	3	B2
	2	24/4	-	-	-
	3	8/5	-	-	-
	4	23/5	24/5	20	A,B,C,D,E,F,L,N
	"	"	28/5	13	A,D,E,N
	5	7/6	8/6	21	A,B,C,D,E,F,G,K,M
	6	26/6	27/6	23	B$_2$,E
	7	5/7	-	-	-
	8	17/7	18/7	10	B$_2$,E
	9	31/7	31/7	24	B$_2$,E
	10	14/8	14/8	10	B$_2$,E
	"	"	16/8	76	B$_2$,E
B	1	17/4	18/4	10	A,B$_1$,C,D,E,F,L
	2	24/4	-	-	-
	3	1/5	7/5	10	B$_1$,E
	"	"	8/5	12	B$_1$
	"	"	16/5	12	A,B$_1$,C,D,E,F,L
	4	15/5	16/5	20	A,B$_1$,C,D,E,F,L
	5	30/5	3/6	12	A,B$_2$,C,D,E,L
	6	13/6	19/6	21	A,B,C,D,E,G,H,K,L,M
	"	(ex cages)	24-27/6	73	B$_2$,E
	7	26/6	28/6	29	B$_2$,E
	8	11/7	12/7	24	B$_2$,E
C	1	10/4	-	-	-
	2	24/4	2/5	36	A,B,C,D,E,F
	"	"	7/5	10	B$_1$,E
	"	"	8/5	14	G,J
	"	"	10/5	17	B,D
	3	8/5	16/5	30	A,B$_1$,C,D,E,F,L
	4	23/5	24/5	23	A,B$_1$,C,D,E,L
	5	7/6	10/6	43	A,B$_2$,C,D,E,L
	6	21/6	26/6	33	B$_2$,E
	7	5/7	8/7	21	B$_2$,E
	8	19/7	19/7	37	B$_2$,E
"Israeli" strain		15/7	18/7	33	B$_2$,E

Table 9. Stains used for detection of microorganisms in smear preparations of *Amblyseius cucumeris*

Source	Batch[1]	Date received (1992)	Date processed (1992)	No. of slides	Stain used
A	1	12/2	12/2	10	B_2,E
	"	"	various	10	B_2
	2	16/2	16/2	10	B_2,E
	"	"	various	5	B_2
	3	4/3	4/3	10	B_2,E
	"	"	various	18	B_2
	4	11/3	11/3	10	B_2,E
	"	"	various	13	B_2
C	1	14/2	14/2	10	B_2,E
	"	"	various	7	B_2
	2	20/2	-	-	-
	"	"	various	12	B_2
	3	27/2	27/2	10	B_2,E
	"	"	various	11	B_2
	4	5/3	5/3	10	B_2,E
	"	"	various	12	B_2

[1] Second batch of each date are *A. cucumeris* that died during longevity studies.

Table 10. Stains used for detection of microorganisms in smear preparations of *Encarsia formosa*

Source	Batch	Date received (1992)	Date processed (1992)	No. of slides	Stain used
A	1	12/2	19/2	10	B_2,E
	2	26/2	4/3	10	B_2,E
	3	4/3	-	-	-
	4	11/3	18/3	10	B_2,E
C	1	14/2	25/2	10	B_2,E
	2	20/2	-	-	-
	3	27/2	28/2	10	B_2,E
	4	5/3	10/2	10	B_2,E

Table 11. Incidence of microorganism infections in *Phytoseiulus persimilis* (S = stained preparations, E = electron micrographs)

Source	Batch	Microsporidia		Rickettsia		Bacteria		Virus		Fungus		Gregarines	
		S	E	S	E	S	E	S	E	S	E	S	E
"Israeli" strain		X	X	X		X				X			
A	1	X	X	X						X			
	2	▨	X	▨	X	▨				▨			
	3	▨		▨	X	▨	X	▨		▨		▨	
	4	X	X	X									
	5			X									
	6	X		X	X	X				X			
	7	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
	8			X						X			
	9	X		X		X				X			
	10	X		X	X	X	X			X			
B	1			X	X		X			X			
	2	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
	3	X		X						X			
	4			X	X	X				X			
	5			X	X		X						
	6	X		X	X	X	X			X			
	7			X		X				X			
	8	X		X		X				X			
C	1	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
	2			X	X	X	X			X			
	3			X	X	X							
	4			X						X			
	5	X		X	X	X	X			X			
	6			X	X	X							
	7			X	X	X							
	8	X		X		X							

Table 12. Incidence of microorganism infections in *Amblyseius cucumeris* (S = stained preparations, E = electron micrographs).

Source	Batch	Microsporidia		Rickettsia		Bacteria		Virus		Fungus		Gregarines	
		S	E	S	E	S	E	S	E	S	E	S	E
A	1	X		X	X	X						X	
	"	X		X		X				X			
	2	X		X									
	"			X		X							
	3			X		X							
	"			X		X							
	4	X		X								X	
	"			X		X				X		X	
C	1			X									
	"			X		X							
	2	▨		▨	X	▨		▨		▨		▨	
	"			X		X							
	3			X		X							
	"			X		X							
	4					X			X				
	"			X		X							

[1] Second batch on each date are *A. cucumeris* that died during longevity studies.

Table 13. Incidence of microorganism infections in *Tyrophagus putrescentiae* (S = stained preparations, E = electron micrographs)

Source	Batch	Microsporidia		Rickettsia		Bacteria		Virus		Fungus		Gregarines	
		S	E	S	E	S	E	S	E	S	E	S	E
A	1												
	2	X											
	3	X		X					X			X	
	4	X		X		X						X	
C	1												
	2		X		X								
	3	X	X									X	
	4	X	X	X	X	X						X	

Table 14. Incidence of microorganism infections in *Encarsia formosa* (S = stained preparations, E = electron micrographs)

Source	Batch	Microsporidia		Rickettsia		Bacteria		Virus		Fungus		Gregarines	
		S	E	S	E	S	E	S	E	S	E	S	E
A	1			X					X				
	2			X						X			
	3	▨		▨		▨	▨						
	4			X									
C	1			X									
	2	▨		▨		▨	▨						
	3			X									
	4			X									

8 APPENDIX I

8.1 Staining Procedures

A. Giemsa stain with acid hydrolysis.

Objective: To differentiate rickettsia from granulosis virus.
Method:

1. Air dry smear.
2. Fix in methanol for 5 minutes.
3. Hydrolyze in 0.1 M HCl for 2 minutes.
4. Stain with Giemsa solution (3 drops of Giemsa stock solution in 2 mL of distilled water) for 45 minutes.
5. Wash with distilled water.
6. Air dry.
7. Mount in Permount®.
8. Examine with light microscope.

Results: Rickettsia stain red and are strongly colored in contrast to granulosis virus.

B. Giemsa staining following methanol fixation.

Objective: To stain microsporidian spores.
Method 1:

1. Air dry smear.
2. Fix in methanol for 5 minutes, then allow to air dry.
3. Stain with Giemsa solution (1 drop of Giemsa stock solution per mL of distilled water.
4. Wash with distilled water until runoff is clear.
5. Air dry.
6. Mount in Permount®.
7. Examine with light microscope.

Method 2: Stain variation as recommended by J. Maddox.

1. Macerate tissue in invertebrate saline.
2. Smear tissue on coverslip.
3. Air dry smear.
4. Fix in 100% methanol for 10 minutes.
5. Stain in 15-20% aqueous Giemsa for 2 hours; pH 7 (time may vary).
6. Air dry.
7. Mount with stain side down in a non-clearing mounting medium.

Results: Microsporidian spores stain purple.

C. Slow staining Giemsa with acid hydrolysis.

Objective: To stain virus rods and inclusion bodies.
Method:

1. Air dry smear.
2. Fix smear in Bouin Dubosq Brasil fixative for 10 minutes.
3. Wash with 70% ethanol until runoff is clear.
4. Wash with 70% ethanol two more times with an hour between each wash. Do not allow the smear to dry.
5. Wash with distilled water after an hour.
6. Rinse in Giemsa buffer for 5 minutes.
7. Stain in buffered Giemsa overnight.
8. Wash with distilled water.
9. Differentiate with 80% ethanol containing 5% glacial acetic acid (for hydrolysis) until the general colour of the slide disappears to a spotty blue.
10. Air dry.
11. Mount in Permount®.
12. Examine with light microscope.

Results: Virus rods stain red; inclusion bodies stain purple.

D. Acid hydrolysis without methanol fixation.

Objective: To differentiate fat globules from crystals.
Method:

1. Air dry smear.
2. Hydrolyze for 2 minutes using 0.1% HCl.
3. Stain with diluted Giemsa (1 drop Giemsa stock solution per mL of distilled water) for 10 minutes.
4. Wash with distilled water.
5. Air dry.
6. Mount in Permount®.
7. Examine with light microscope.

Results: Fat globules stain purple to red; crystals do not stain.

E. Macchiavello stain.

Objective: To differentiate rickettsia, crystals, and spheroidocytes from bacteria and albuminoid crystals.

Method:

1. Air dry smear.
2. Fix in methanol for 5 minutes.
3. Stain for in 1% basic fuchsin for 60 minutes.
4. Wash in distilled water until runoff is clear.
5. Differentiate with 0.5% citric acid for 5 seconds.

6. Wash with distilled water.
7. Counterstain with 1% aqueous methylene blue for 20 seconds.
8. Air dry.
9. Mount in Permount®.
10. Examine with light microscope.

Results: Rickettsia, crystals, and spheroidocytes stain red; bacteria and albuminoid crystals stain blue. Cell nuclei stain blue.

Note: Two variations of this stain are as follows:

1. **EII:** Slides were placed in a bath of basic fuchsin for 1 hour.
2. **EIII:** Slides were heated approximately 15 cm above an alcohol lamp, allowing the basic fuchsin to steam for 45 seconds. The solution was replaced at intervals to prevent drying of the smear.

F. Guegin's stain.

Objective: To differentiate virus from host fat cells and fungi.

Method:

1. Air dry smear.
2. Stain with lactophenol methylene blue for 10 minutes.
3. Wash with distilled water until runoff is clear.
4. Stain with Sudan III for 10 minutes.
5. Wash with distilled water.
6. Air dry.
7. Mount in Permount®.
8. Examine with light microscope.

Results: Host fat cells stain yellow; fungi stain blue.

G. Gram stain.

Objective: To differentiate Gram positive bacteria from Gram negative bacteria.

Method:

1. Air dry smear.
2. Heat fix by passing through flame.
3. Place slides on a staining rack and flood with ammonium oxalate and crystal violet for 1 minute.
4. Rinse in running tap water for 5 seconds.
5. Rinse slide with Gram's iodine and then flood slide for 1 minute.
6. Rinse in running tap water for 5 minutes.
7. Pass the wet slide through three changes of n-propyl alcohol in separate coplin jars for 1 minute each.
8. Rinse in running tap water for 5 seconds.
9. Rinse slide with safranin counterstain; then flood for 1 minute.
10. Rinse in running tap water for 5 seconds.
11. Air dry.
12. Mount in Permount®.
13. Examine with light microscope.

Results: Gram positive organisms stain blue-violet; Gram negative organisms stain red.

H. Papanicolaou stain.

Objective: To differentiate cellular components.
Method:

1. Spray wet smear with Cytoprep® to fix smear.
2. Stain with Harris' haematoxylin for 4 minutes.
3. Wash in running tap water for 5 minutes.
4. Differentiate with two quick dips in dilute HCl.
5. Wash in running tap water for 5 minutes.
6. 10 dips in each of 50%, 70%, 80%, 95% ethanol.
7. Stain in Harris' haematoxylin for 2 minutes.
8. 10 dips in 3 changes of 95% ethanol.
9. Stain in working solution of light green, Bismark brown, and eosin 36 for 3 minutes.
10. 10 dips in 3 changes of 95% ethanol.
11. 10 dips in 2 changes of absolute ethanol.
12. Xylene for 2 minutes; change for 5 minutes.
13. Mount in Permount®.

Results: Nuclear material stains blue with clear detail; cytoplasm stains various shades of green, pink, yellow, and grey.

I. Ziehl-Nielsen acid fast stain.

Objective: To differentiate acid fast bacteria from non-acid fast bacteria.

Method:

1. Air dry smear.
2. Fix in 10% formalin.
3. Stain in Zeihl's carbol fuchsin for 30 minutes.
4. Wash in tap water.
5. Decolorize in acid alcohol until smear is pale pink.
6. Wash with tap water.
7. Stain in saturated aqueous methylene blue for 5 to 15 seconds.
8. Wash with tap water.
9. Air dry.
10. Mount in Permount®.
11. Examine with light microscope.

Results: Acid fast organisms stain red; non-acid fast organisms stain blue.

J. Periodic acid Schiff stain.

Objective: To demonstrate presence of mucous proteins.
Method:

1. Air dry.

2. Place in periodic acid for 5 minutes.
3. Wash in running tap water for 5 minutes.
4. Place in Schiff's reagent for 10 minutes.
5. Wash in distilled water.
6. Air dry.
7. Mount in Permount®.
8. Examine with light microscope.

Results: Mucous proteins stain red.

K. Neutral red stain.

Objective: To demonstrate the presence of rickettsia.
Method:

1. Air dry smear.
2. Stain in neutral red for 10 to 15 minutes.
3. Rinse in tap water.
4. Air dry.
5. Mount in Permount®.
6. Examine with light microscope.

Results: Rickettsia stain red.

L. Lacto-aceto-orcein stain.

Objective: To stain the nuclei within spores, particularly to determine the number of nuclei present. To demonstrate microsporidia in the sporophorous vesicle stage.
Method:

1. Air dry smear.
2. Stain in lacto-aceto-orcein for 2 minutes.
3. Rinse in tap water.
4. Air dry.
5. Mount in Permount®.
6. Examine with light microscope.

M. Masson's trichome stain.

Objective: Tissue differentiation.
Method:

1. Air dry smear.
2. Fix in Bouin's fixative overnight.
3. Wash in running tap water until runoff is clear.
4. Stain in Weigert's haematoxylin for 4 minutes.
5. Wash in running tap water for 10 minutes.
6. Rinse in distilled water.
7. Stain in Biebrecht scarlet/acid fuchsin for 5 minutes.
8. Rinse in distilled water.
9. Stain in phosphomolybdic/phosphotungstic acid for 3 minutes.

10. Stain in aniline blue solution for 4 minutes.
11. Rinse in distilled water.
12. Differentiate in 1% acetic acid for 3 to 5 minutes.
13. 95% ethanol; 2 changes of absolute ethanol.
14. 2 changes of xylene.
15. Mount with Permount®.
16. Examine with light microscope.

Results: Nuclei stain black; collagen and mucous stain blue; cytoplasm, keratin, and muscle fibres stain red.

N. India ink stain.

Objective: Measurement of microsporidian spores.
Method:

1. Smear tissue in 50% solution of India ink.
2. Spread 5 µL drop of India ink and crushed tissue on microscope slide and allow to air dry.
3. Mount with Permount® and examine with light microscope.

9 APPENDIX II

9.1 Preparation of Samples for Transmission Electron

1. Pick up live mites individually with a fine paintbrush and place into Universal fixative (1% formaldehyde, 1.5% glutaraldehyde in 0.12M cacodylate buffer, pH 7.2).

2. Place container under slight vacuum (at room temperature) for one to several hours. Some samples will sink, others will remain afloat.

3. Store samples in a refrigerator until they are processed. Samples may remain in fixative from 24 hours to several days.

4. Pipette off fixative and replace with 0.12M cacodylate buffer, pH 7.2. Change buffer 3 times. (Total time in buffer is 1 hour).

5. Postfix samples in 1% osmium tetroxide, in 0.12M cacodylate buffer, pH 7.2 for 2 hours.

6. Dehydrate and infiltrate as follows:

distilled water	5-10 minutes
50% ethanol	30 minutes
70% ethanol	30 minutes
90% ethanol	30 minutes
100% ethanol	30 minutes
ethanol:propylene oxide (1:1)	30 minutes
propylene oxide	60 minutes
propylene oxide:Spurrs	minimum of overnight
pure Spurrs (epoxy resin)	minimum of 24 hours

Note: All steps include a minimum of three changes in each solution.

7. Put samples into flat moulds (one per mould) and align as desired.

8. Harden blocks in a 60 degree oven for a minimum of 16 hours.

10 APPENDIX III

10.1 Plates

Magnification in Plates 4-16 is ~ x 3,200 unless otherwise indicated.

10.1.1 Plate 1

Fig. 1. Normal coloration of *Phytoseiulus persimilis* (adult female).

Figs. 2-6. Abnormal coloration in *P. persimilis*, showing "white gut" in region of Malpighian tubules and speckling or larger black dots in rear of abdomen. Large ovoid body in Fig. 5 is a predator egg.

Plate 1

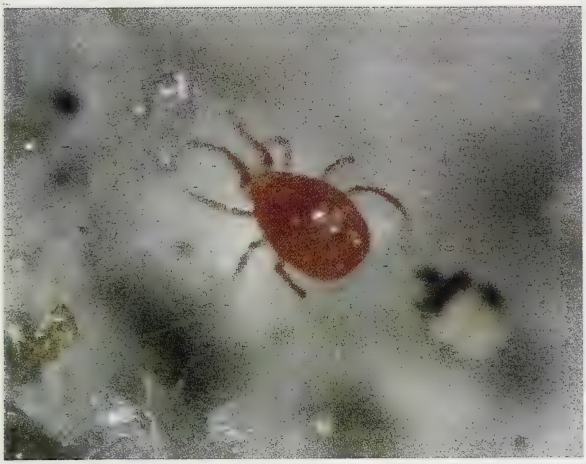
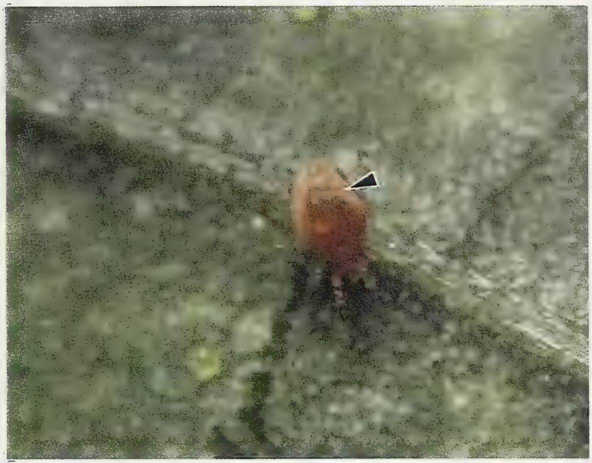


Fig. 1.



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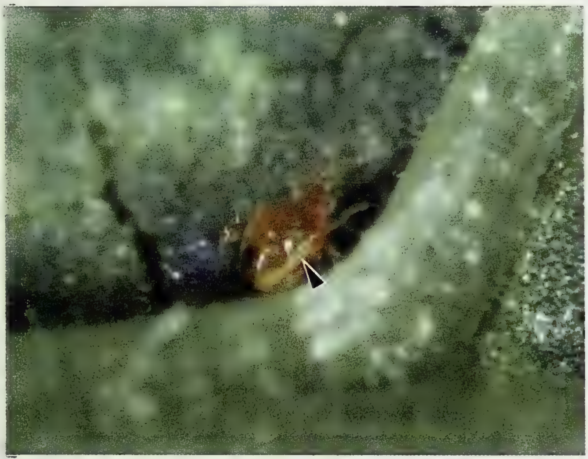

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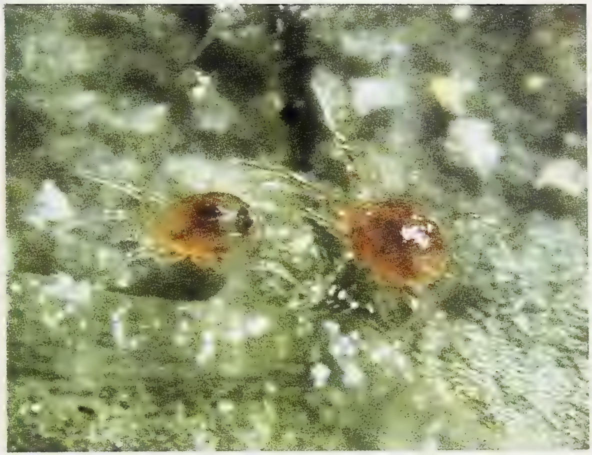

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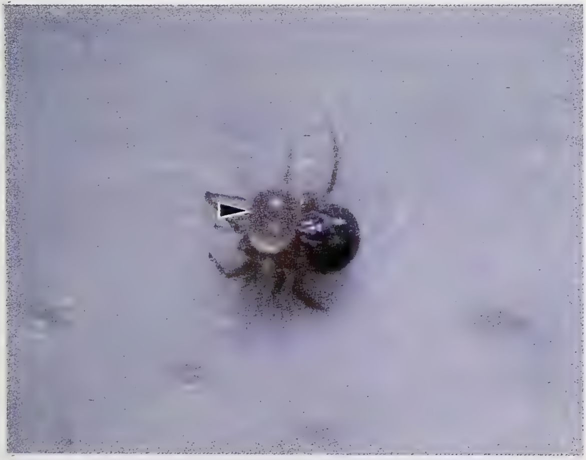

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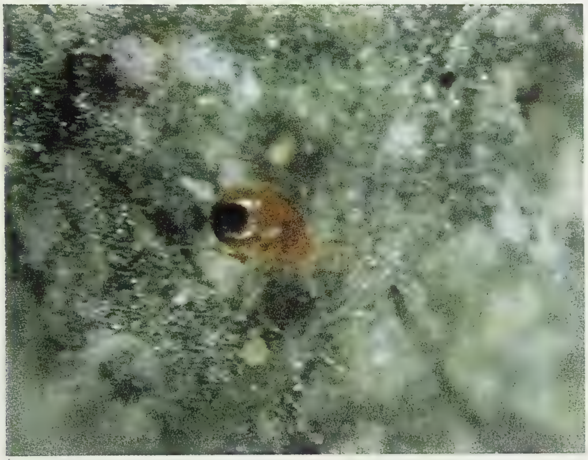


Fig. 6.

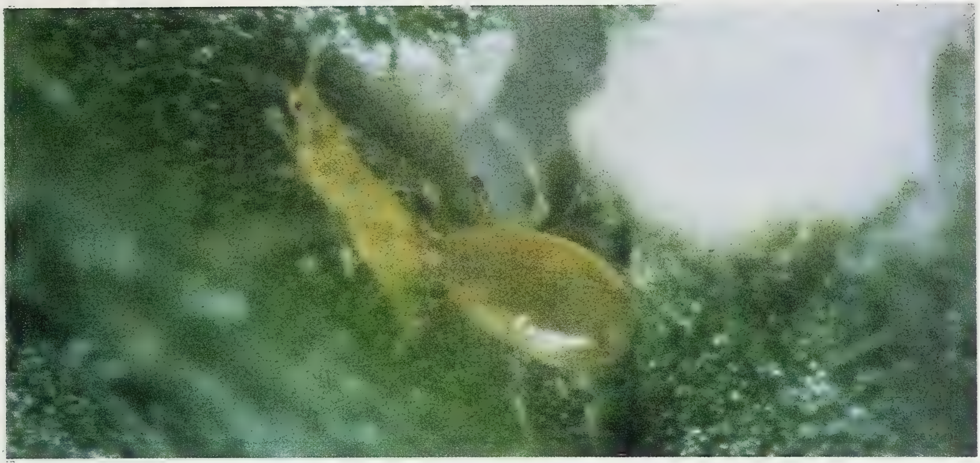
10.1.2 Plate 2

Fig. 1. *Amblyseius cucumeris* feeding on thrips larva.

Fig. 2. *Encarsia formosa* laying eggs in early nymphal stages of whitefly.

Fig. 3. Parasitized whitefly "scales" glued on cardboard. Note small, circular holes in scales which indicate successful emergence of adult *E. formosa.*

Plate 2



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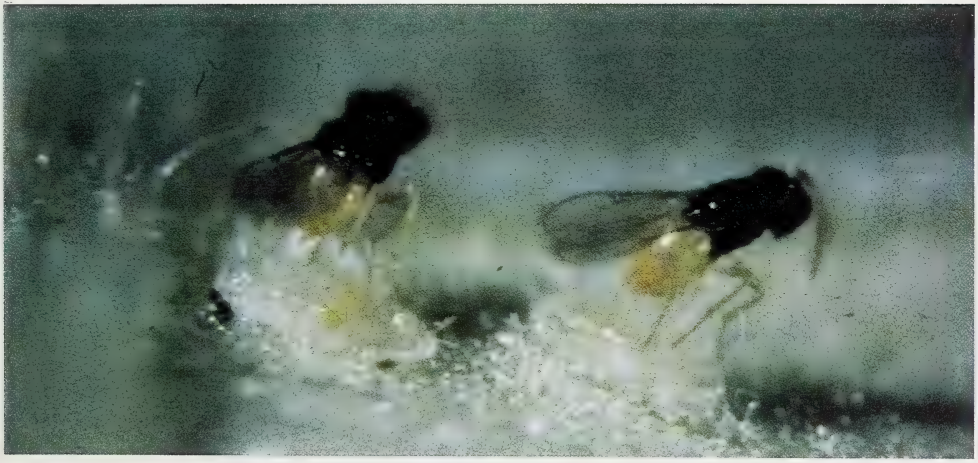

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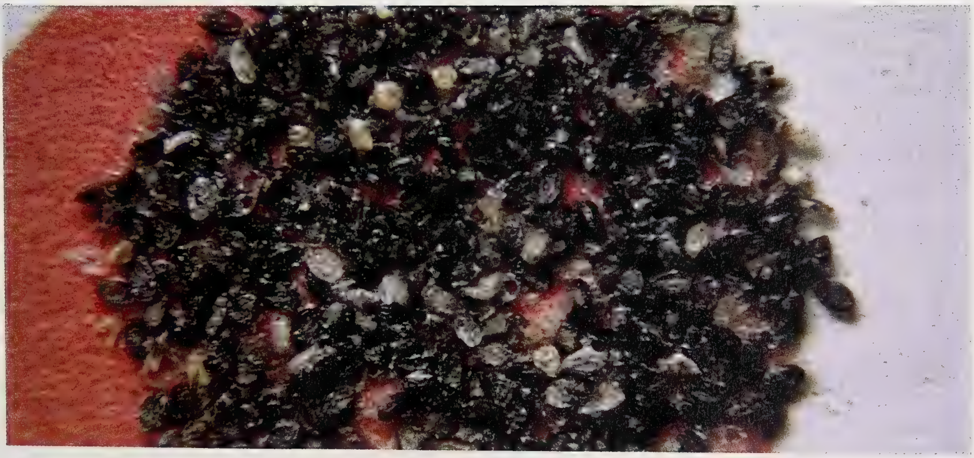


Fig. 3.

10.1.3 Plate 3

Fig. 1. *Phytoseiulus persimilis* cleared in polyvinyl alcohol to show presence of foreign material in Malpighian tubules and rectum that is responsible for "white gut" symptoms.

Fig. 2. Screened cage surrounded by soapy water used to rear and isolate cultures of spider mites and *P. persimilis*.

Fig. 3. Rearing apparatus used to determine longevity and fecundity of *P. persimilis* and *A. cucumeris*. Small screened dishes held bean leaf discs with spider mite or thrips on moist felt. One predator was placed in each dish and dishes were everted over wet paper in a larger container and maintained at 25°C for 7 days.

Plate 3

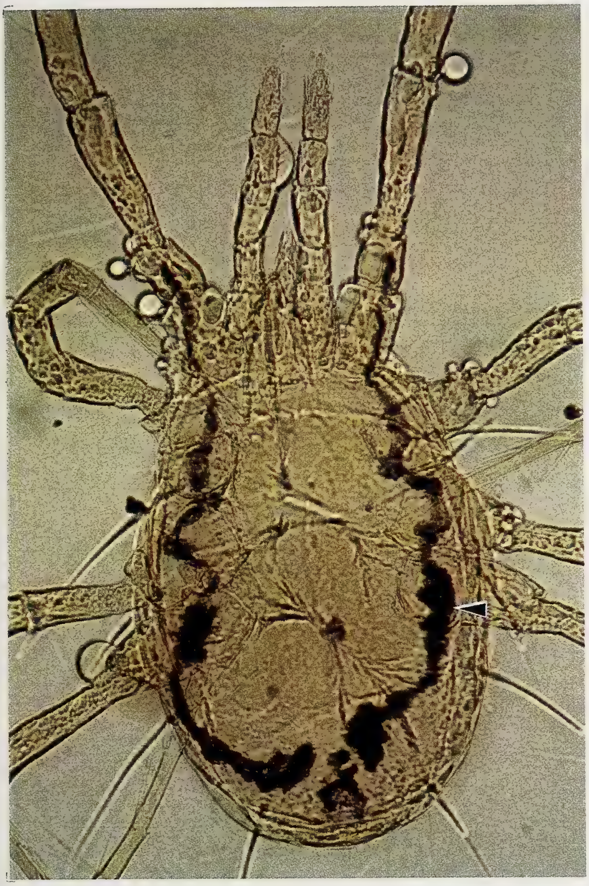
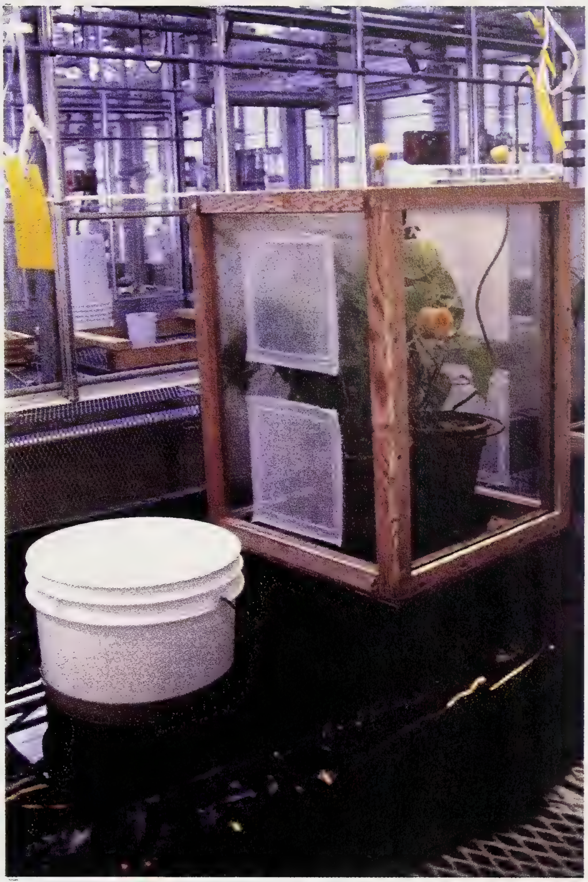


Fig. 1



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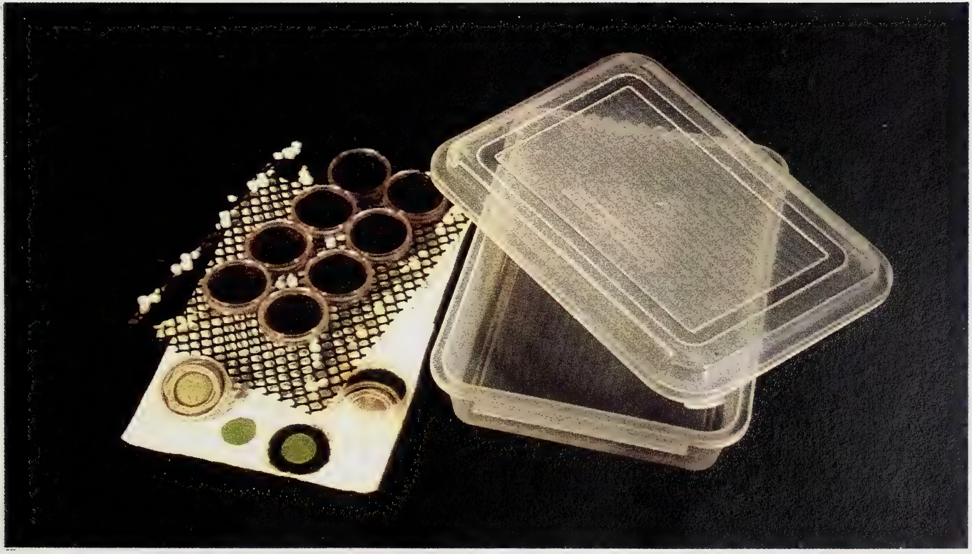


Fig. 3.

10.1.4 Plate 4

Figs. 1-6. Stages of microsporidia in smeared and stained preparations of *Phytoseiulus persimilis.*

Fig. 1. Spores of microsporidium; Source A, batch 4. Stained with India ink after air drying. Note background of faintly stained coccoid forms.

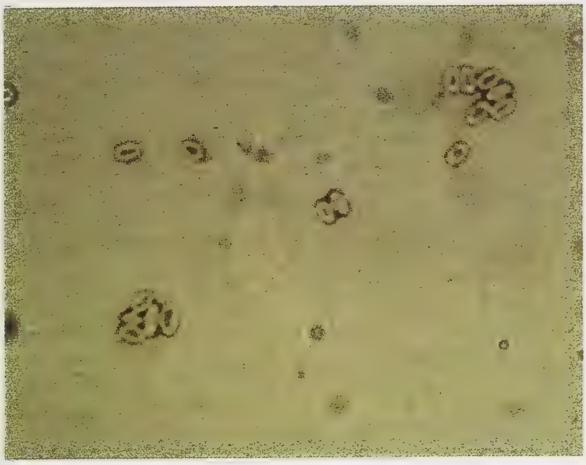
Fig. 2. Spores of microsporidium; Source A, batch 4. Stained with Giemsa after HCl hydrolysis (Stain A). Note apparently dividing or binucleate stages.

Fig. 3. Spores of microsporidium; Source A, batch 4. Stained with Giemsa after methanol fixation, (Stain B, method 1). Note also pink coccoid forms, possibly rickettsia.

Fig. 4. Spores of microsporidium; source shipped as "Israeli" strain of *P. persimilis.* Stained with Giemsa after methanol fixation (Stain B, method 2). Note remains of sporophorous vesicle (left) and sporoblast or sporoplasm (centre).

Fig. 5. Spores and sporogonial stages of a microsporidian; Source A, batch 6 (Stain B, method 2).

Fig. 6. Microsporidian spores with everted polar flagella (centre): Source A, batch 6 (Stain B, method 2).



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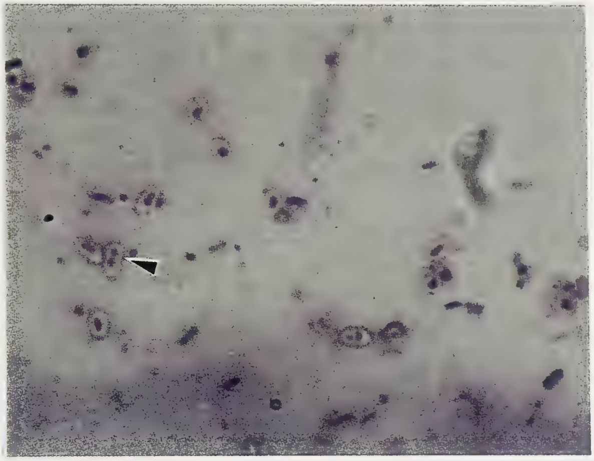

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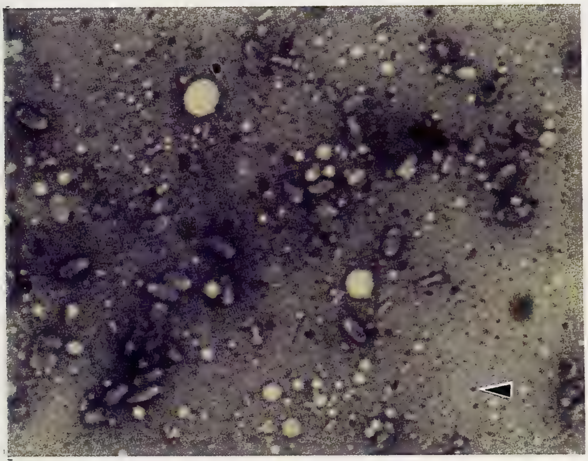

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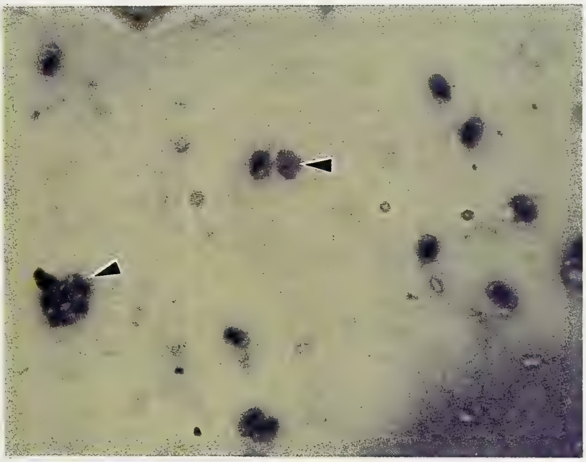

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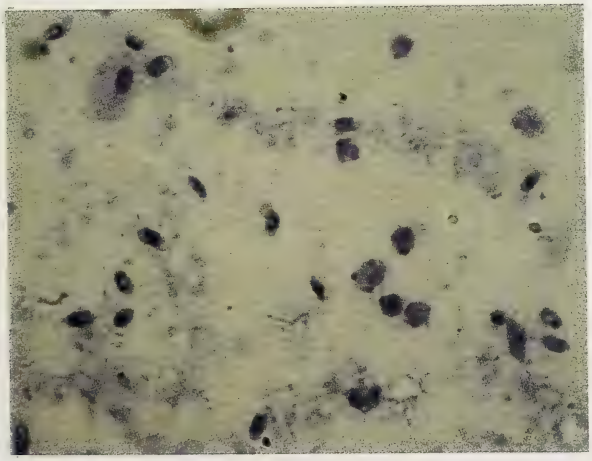

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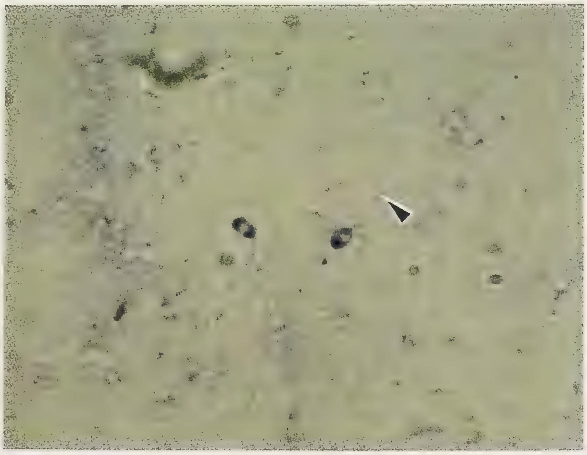


Fig. 6.

10.1.5 Plate 5

Figs. 1-6. Stages of microsporidia in smeared and stained preparations of *Phytoseiulus persimilis.*

Fig. 1. Spores and sporophorous vesicle containing many developing spores; Source "Israeli" strain. Stained with Giemsa after methanol fixation (Stain B, method 2). Note also pink stained rickettsia.

Fig. 2. Spores and various sizes of sporophorous vesicles, Source "Israeli" strain (Stain B, method 2).

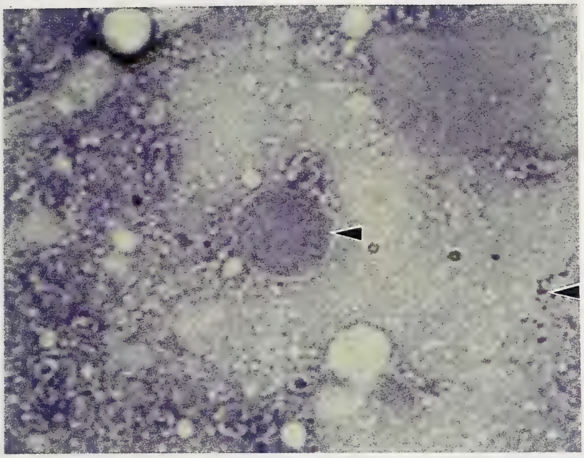
Fig. 3. Merogonial stages in protonymph; Source A, batch 10 (Stain B, method 2). Note also elongate pink bodies, possibly rickettsia.

Fig. 4. Merogonial stages in protonymph; Source A, batch 10 (Stain B, method 2) (x 5,000).

Fig. 5. Merogonial stages in egg of *Phytoseiulus persimilis*; Source B from cages (Stain B, method 2).

Fig. 6. Spores and sporophorous vesicle: Source C, batch 8 (Stain B, method 2).

Plate 5



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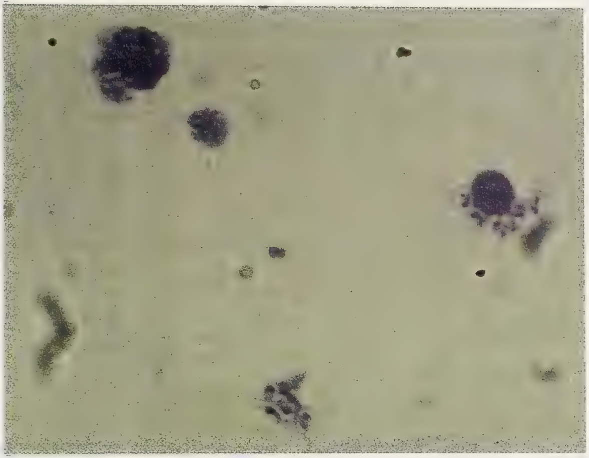

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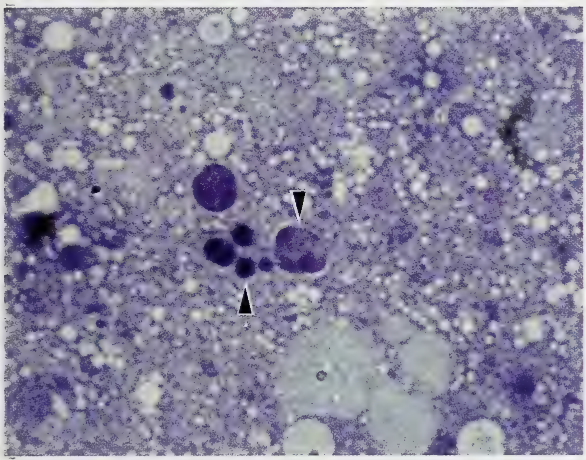

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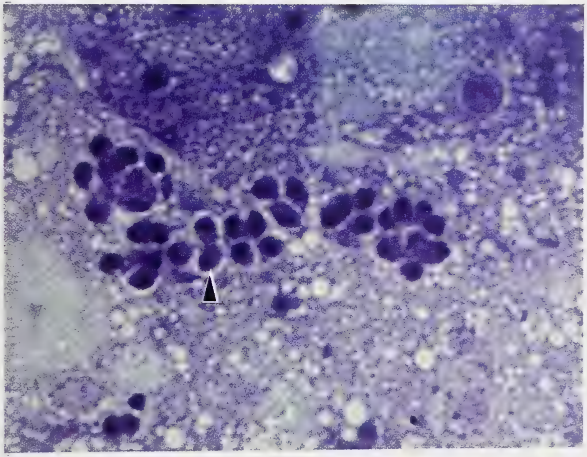

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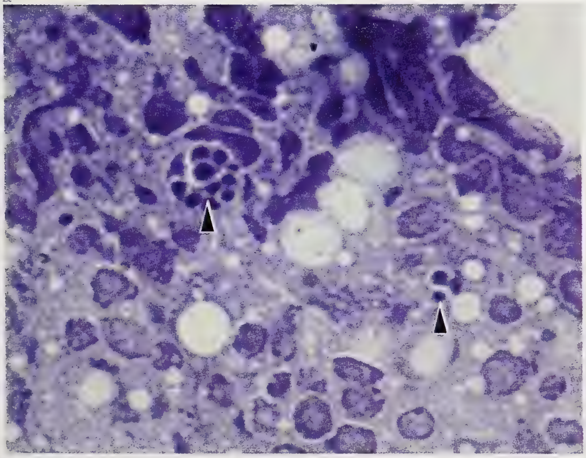

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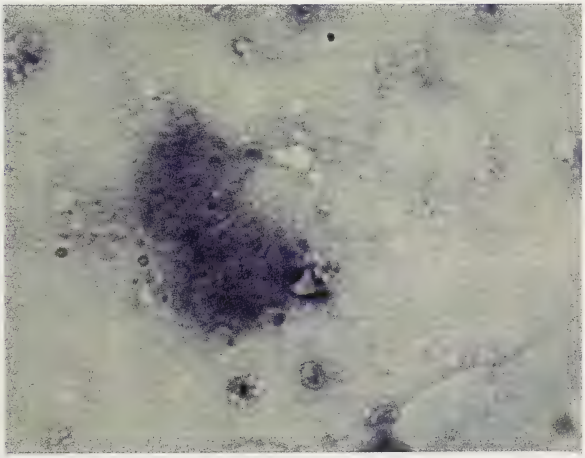


Fig. 6.

10.1.6 Plate 6

Figs. 1-6. Coccal forms of microorganisms in smeared and stained *Phytoseiulus persimilis*, suspect rickettsia.

Fig. 1. Heavy coccal infection in protonymph; Source A, batch 10 (Stain B, method 2). Note also larger pink spheres and much larger fungal spores.

Fig. 2. Single and paired cocci; Source A, batch 10. Macchiavello stain (Stain E).

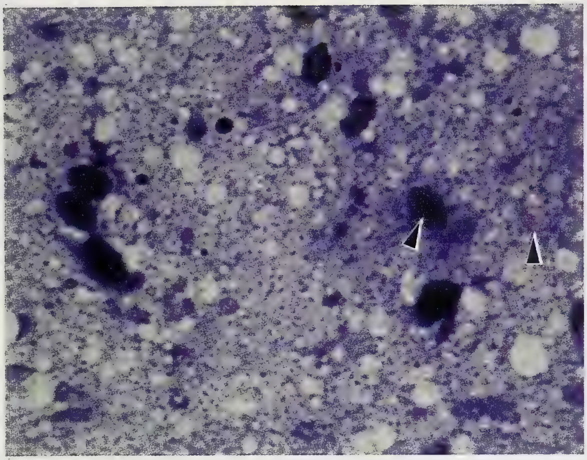
Fig. 3. Crystal-forming rickettsia and elongate forms within vacuoles in protonymph; Source A, batch 10 (Stain B, method 2).

Fig. 4. Crystal-forming rickettsia, coccoid forms, and vacuoles possibly containing rickettsia, Source B, batch 3 (Stain B, method 1).

Fig. 5. Single and paired cocci, and vacuole containing short rods; Source A, batch 10 (Stain B, method 2) (x 5000).

Fig. 6. Heavy coccal infection in adult; Source A, batch 6 (Stain B, method 2).

Plate 6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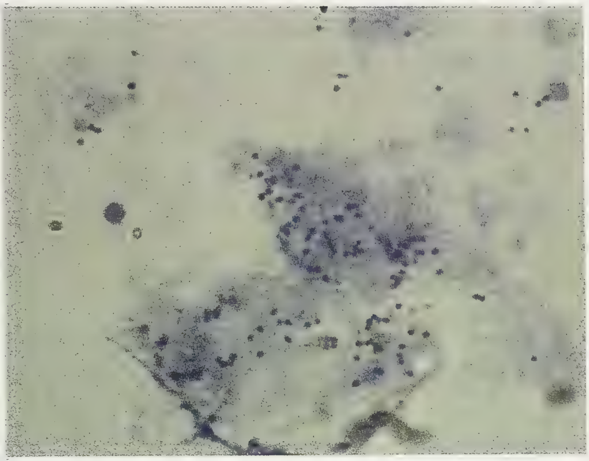
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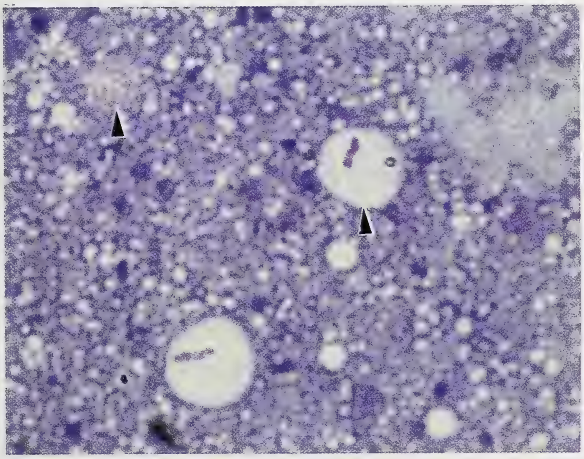
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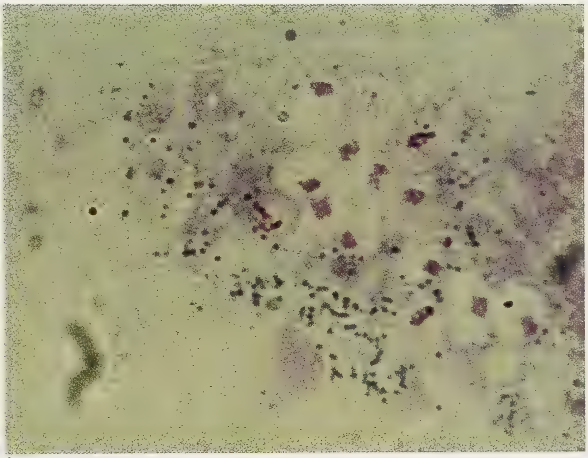
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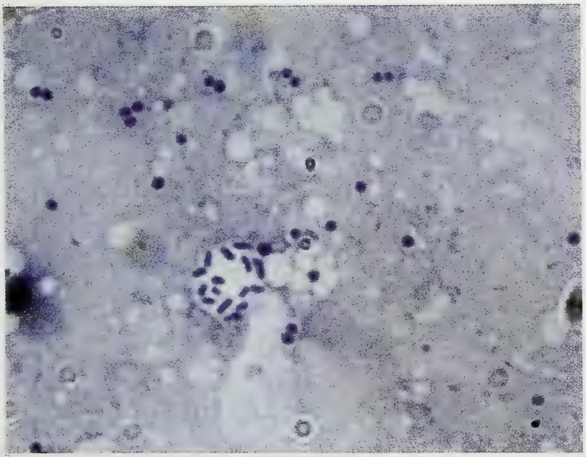
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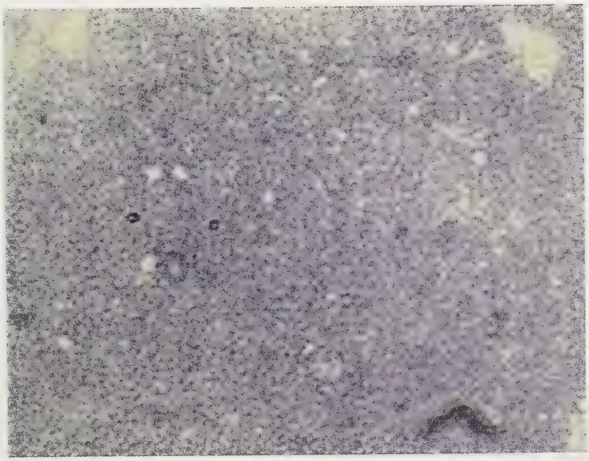


Fig. 6.

10.1.7 Plate 7

Figs. 1-6. Coccal and crystal-forming stages of microorganisms in smeared and stained *Phytoseiulus persimilis*, suspect rickettsia.

Fig. 1. Coccal forms, some appearing birefringent; Source C, batch 4. Macchiavello Stain (Stain E).

Fig. 2. Drift of coccal forms from crystal-containing cell; Source C, batch 5. Stained with Giemsa after HCl hydrolysis (Stain A).

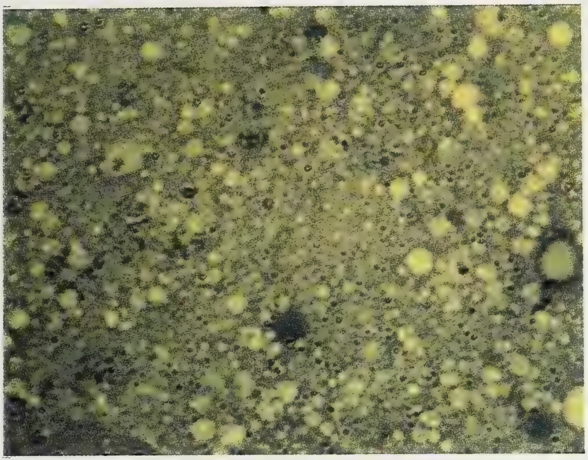
Fig. 3. Coccal forms; Source B, batch 3 (Stain B, method 1).

Fig. 4. Coccal forms and crystals; Source B, batch 4 (Stain B, method 1).

Fig. 5. Crystal-forming rickettsia in deuteronymph; Source B, from cages (Stain B, method 2).

Fig. 6. Suspect crystal-forming rickettsial stages; Source B, batch 3 (stained with Giemsa after overnight fixation in Bouin's fixative).

Plate 7



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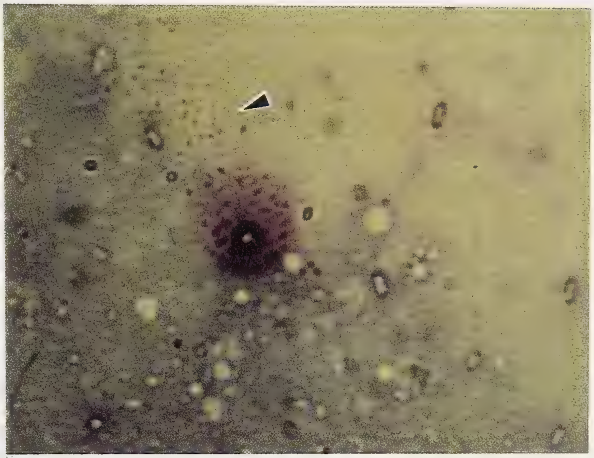

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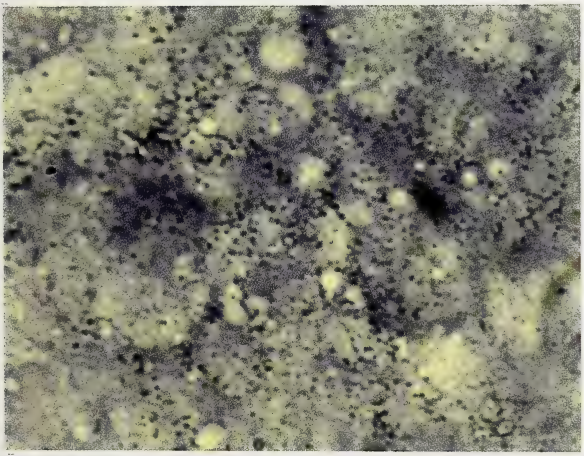

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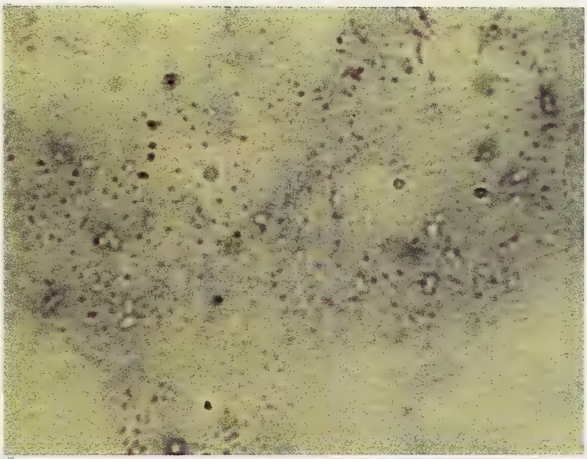

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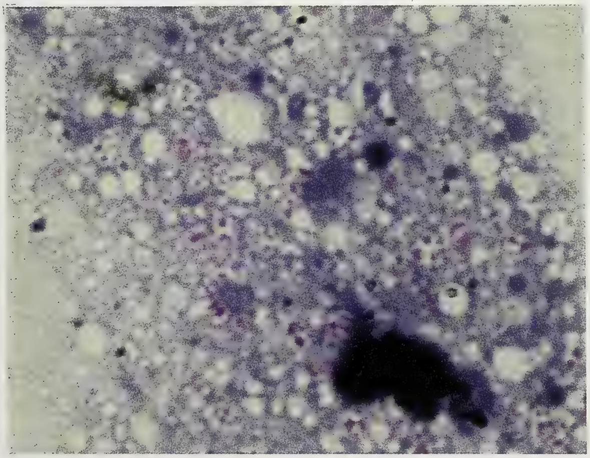

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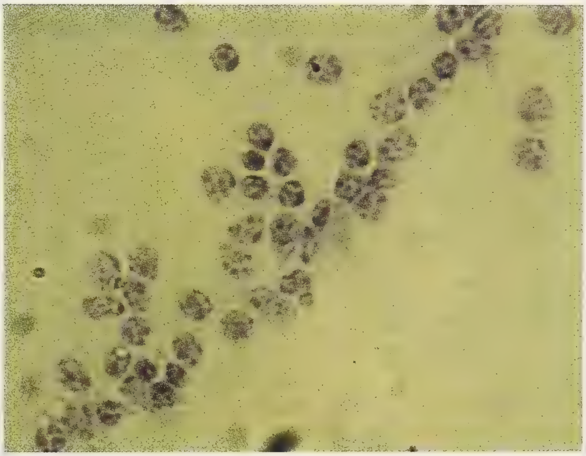


Fig. 6.

10.1.8 Plate 8

Figs. 1-6. Various microorganisms and inclusion bodies in smeared and stained *Phytoseiulus persimilis*.

Fig. 1. Fungal stages, possibly *Entomophaga* sp.; Source "Israeli" strain. Stained with Giemsa after methanol fixation (Stain B, method 2).

Fig. 2. As Fig. 1; Source A, Batch 10 (Stain B, method 2).

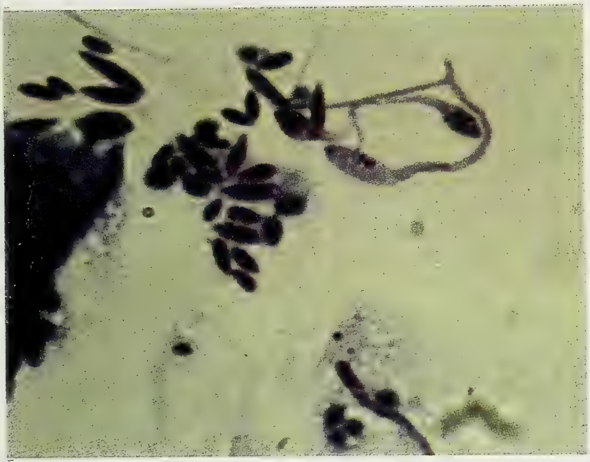
Fig. 3. Fungal mycelium; Source C, batch 5 (mite died). Stained by Guegin's method (Stain F). Note also microsporidian spores and cocci.

Fig. 4. Multiple infection with microsporidia, fungus, rickettsia, and possibly bacteria; Source "Israeli" strain (Stain B, method 2).

Fig. 5. Developing microsporidia and unknown microorganism in protonymph; Source B from cages (Stain B, method 2).

Fig. 6. Crystal similar to those reported from citrus red mite infected with virus; Source B, batch 3. Giemsa stain after air drying.

Plate 8



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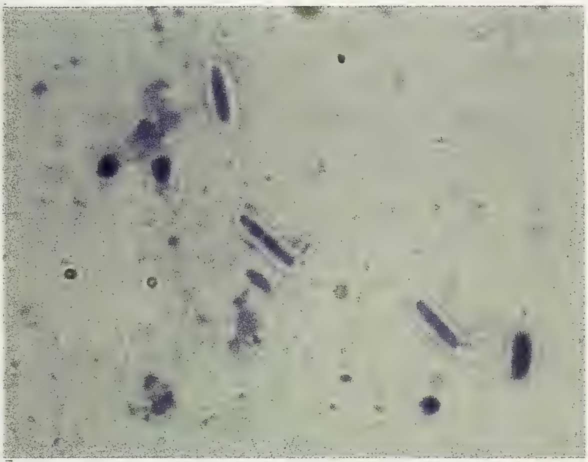

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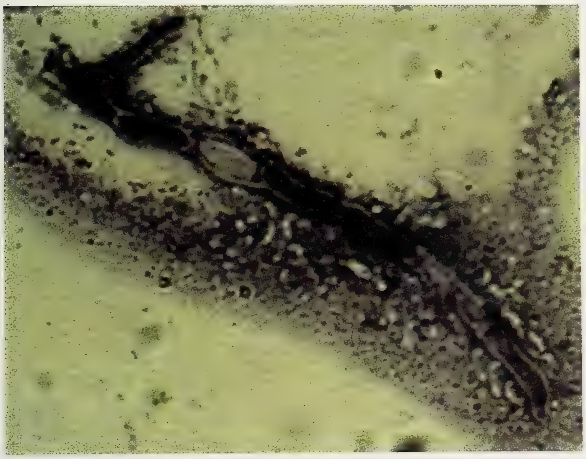

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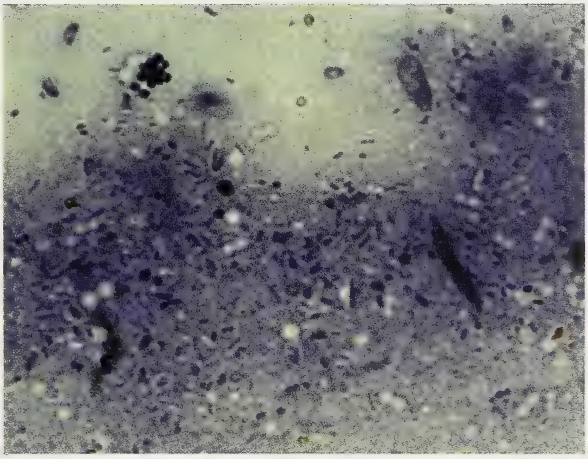

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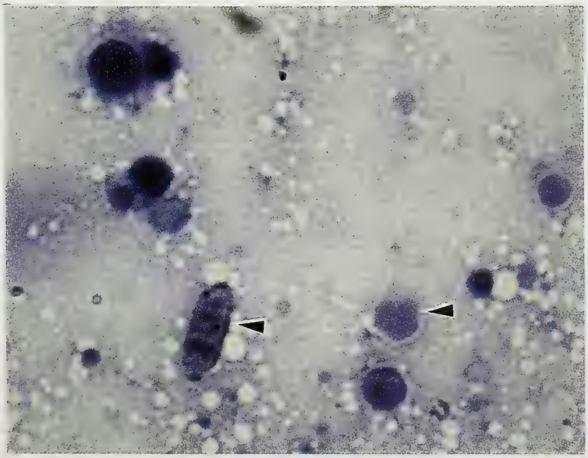

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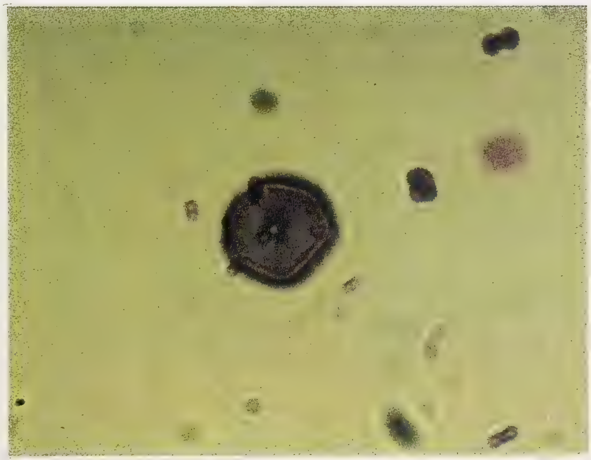


Fig. 6.

10.1.9 Plate 9

Figs. 1-6. Bacteria-like microorganisms in smeared and stained *Phytoseiulus persimilis*.

Fig. 1. *Bacillus* spores (rare): Source B, batch 4. Stained with Giemsa after methanol fixation (Stain B, method 1).

Fig. 2. Short rod and coccal forms; Source C, batch 2 (Stain B, method 1).

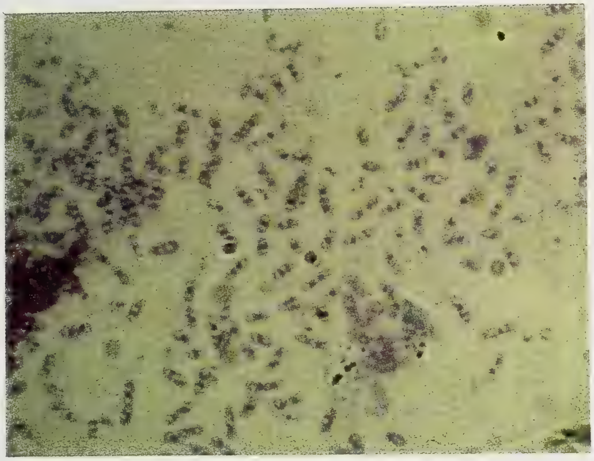
Fig. 3. Short rods and bipolar forms; Source C, batch 5. Macchiavello stain (Stain E II).

Fig. 4. Bipolar rods similar to those in Fig. 3; Source B, batch 4. Stained with Giemsa after slow HCl hydrolyzation (Stain D).

Fig. 5. Single and paired coccoid forms; Source C, batch 5. Stained with Giemsa after HCl hydrolysis (Stain A).

Fig. 6. Coccal forms; Source C, batch 5. (Stain D).

Plate 9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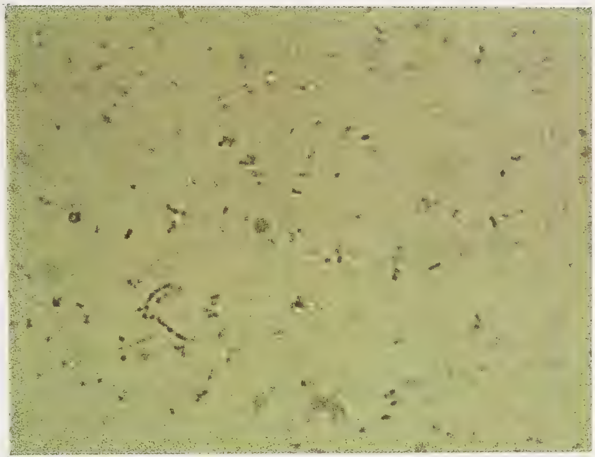
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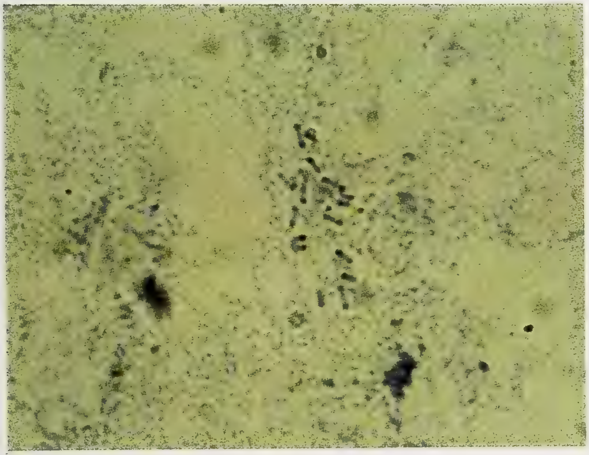
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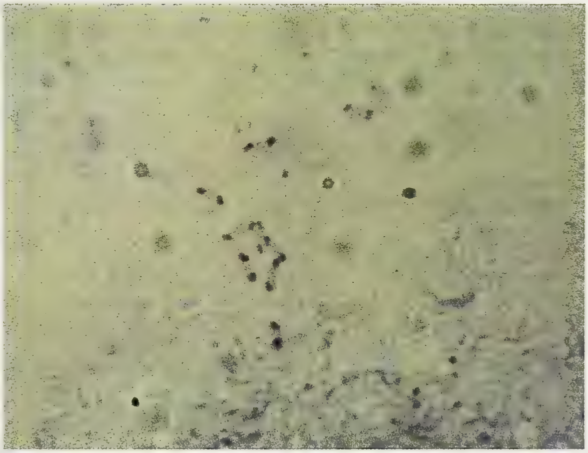
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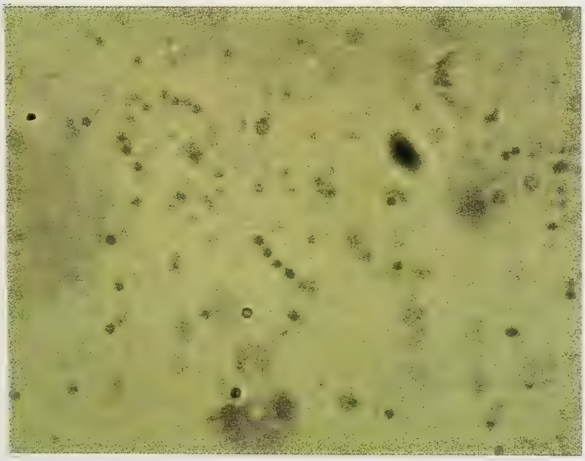
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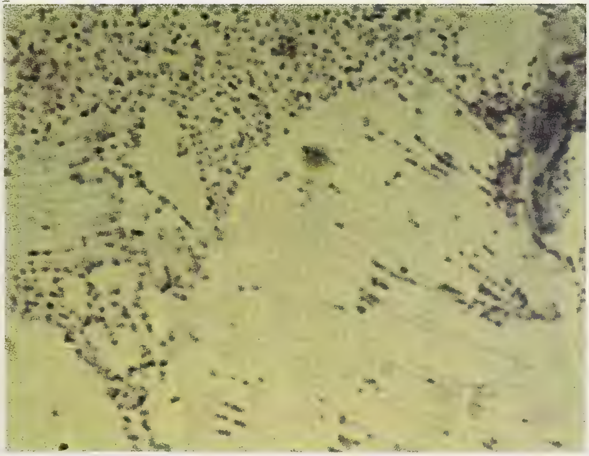


Fig. 6.

10.1.10 Plate 10

Figs. 1-6. Miscellaneous artifacts in smeared and stained *Phytoseiulus persimilis*.

Fig. 1. Unidentified. In egg; Source C from cages. Giemsa stain. Note also variously-stained spore-like objects.

Fig. 2. Pink structures similar in appearance to those in Fig. 1; Source A, batch 9. Stained with Giemsa after methanol fixation (Stain B, method 2). Note also bacteria-like rods.

Fig. 3. Rickettsia (red) and unstained dumbbell-shaped bodies believed to be calcium crystals; Source C, batch 2 (Stain B).

Fig. 4. Dumbbell-shaped bodies, believed to be calcium crystals; Source C, batch 2. (Stain B).

Fig. 5. As in Fig. 4; Source C, batch 5. Stained with Giemsa after HCl hydrolysis (Stain A).

Fig. 6. As in Fig. 4; Source C, batch 5. Macchiavello stain (Stain E).

Plate 10

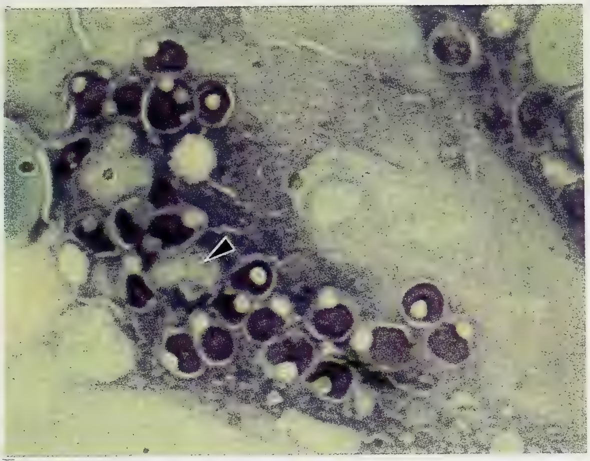


Fig. 1.

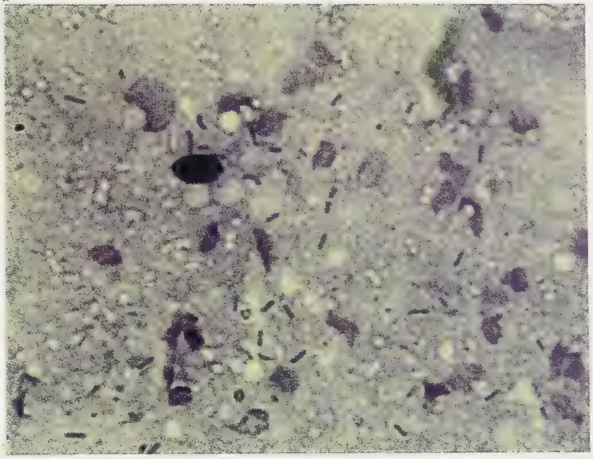
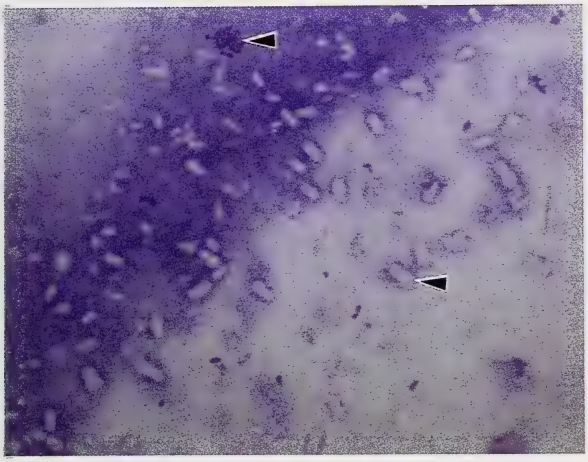


Fig. 2.



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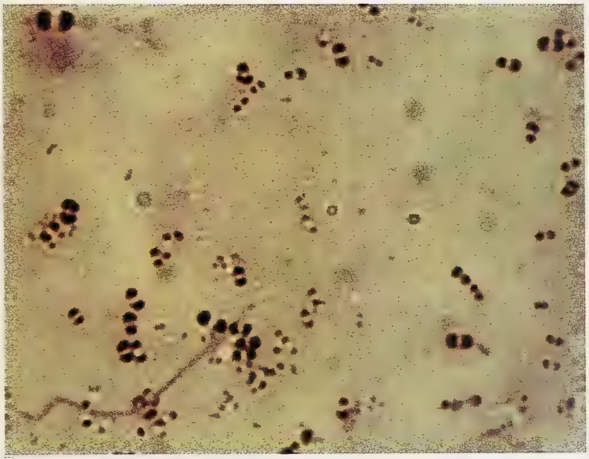

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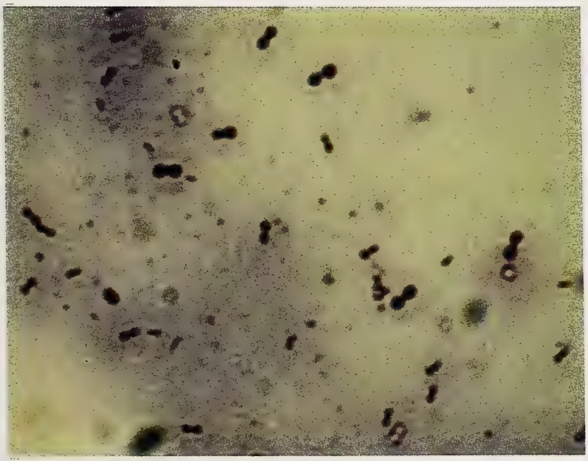

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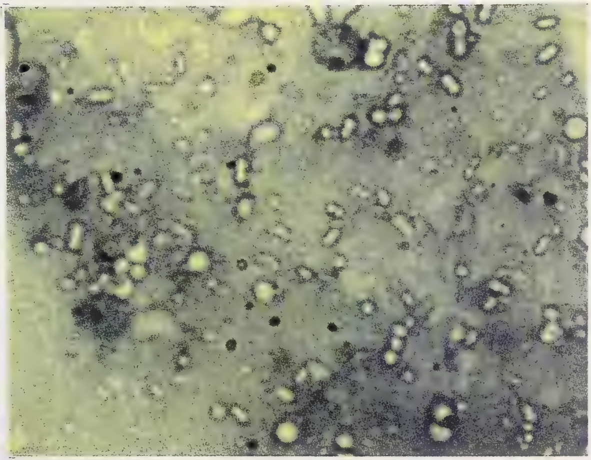


Fig. 6.

10.1.11 Plate 11

Figs. 1-6. Microorganisms found in smeared and stained *Amblyseius cucumeris*.

Fig. 1. Microsporidian spores; Source A, batch 1. Macchiavello stain (Stain E).

Fig. 2. Suspect microsporidian stage; Source C, batch 4 (mite died). Stained with Giemsa after methanol fixation (Stain B, method 2).

Fig. 3. Rickettsia (pink); Source A, batch 3 (Stain B, method 2).

Fig. 4. Cell (nucleus at centre) infected with rickettsia/bacteria; Source A, batch 4 (mite died) (Stain B, method 2).

Fig. 5. Birefringent crystals; Source C, batch 3 (mite died) (Stain B, method 2).

Fig. 6. Unidentified; Source A, batch 1. Macchiavello Stain (Stain E).

Plate 11

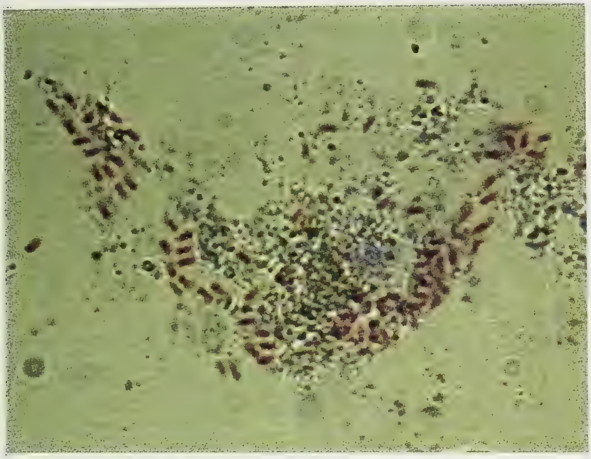
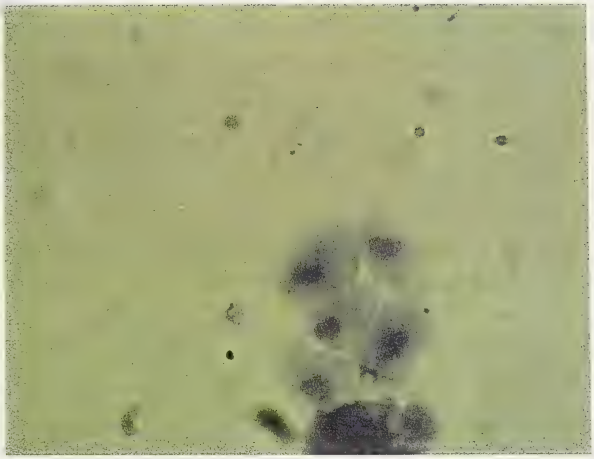


Fig. 1.



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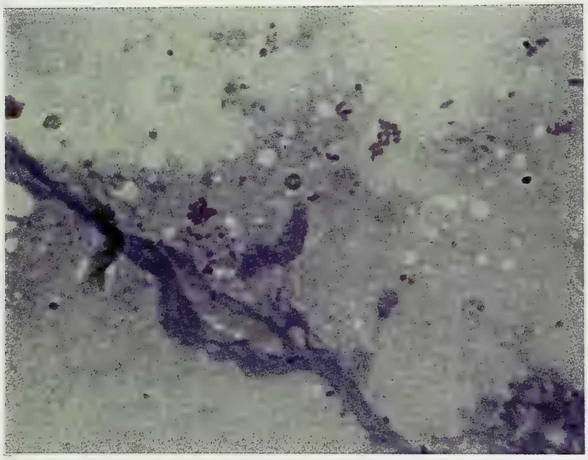

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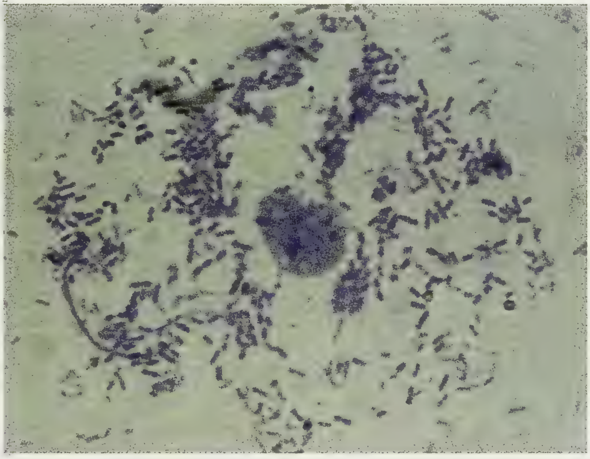

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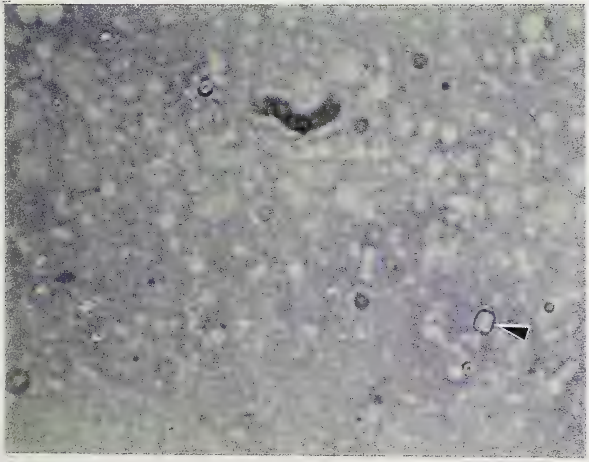

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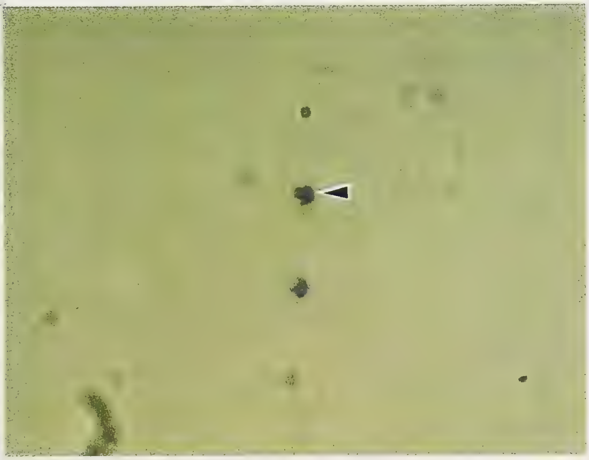


Fig. 6.

10.1.12 Plate 12

Figs. 1-6. Miscellaneous microorganisms found in smeared and stained *Amblyseius cucumeris*.

Fig. 1. Diplococci; Source C, batch 2 (mite died). Stained with Giemsa after methanol fixation (Stain B, method 2).

Fig. 2. Diplococci; Source A, batch 1. Macchiavello stain (Stain E). Note also microsporidia spore (red).

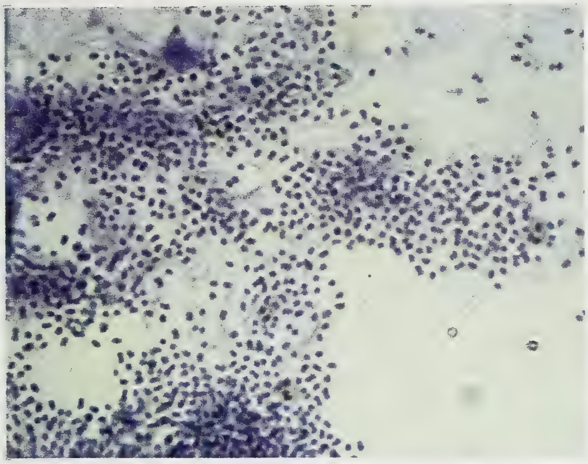
Fig. 3. Heavy infection with coccoid forms; Source C, batch 3 (mite died) (Stain B, Method 2).

Fig. 4. Mixed infection, rod-like and coccoid forms; Source A, batch 1 (mite died) (Stain B, method 2).

Fig. 5. Bacterial rods; Source C, batch 2 (mite died) (Stain B, method 2).

Fig. 6. Yeast-like microorganisms; Source C, batch 3 (Stain B, method 2) (Photo courtesy of B.A. Keddie).

Plate 12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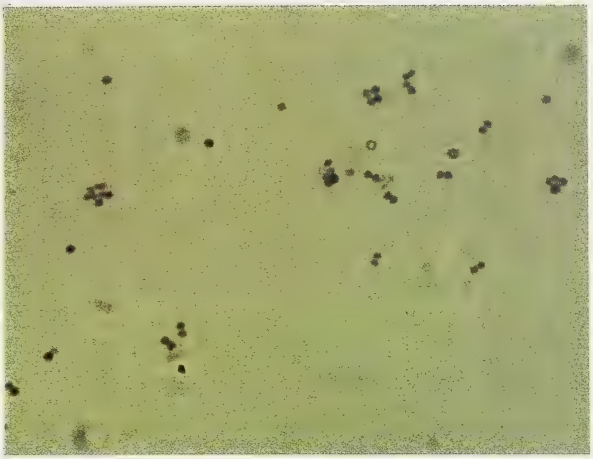
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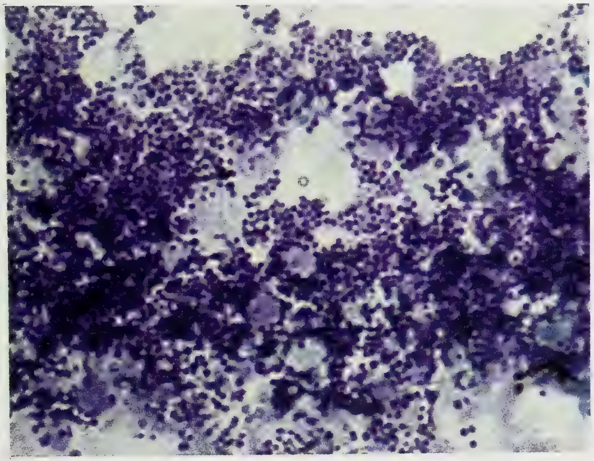
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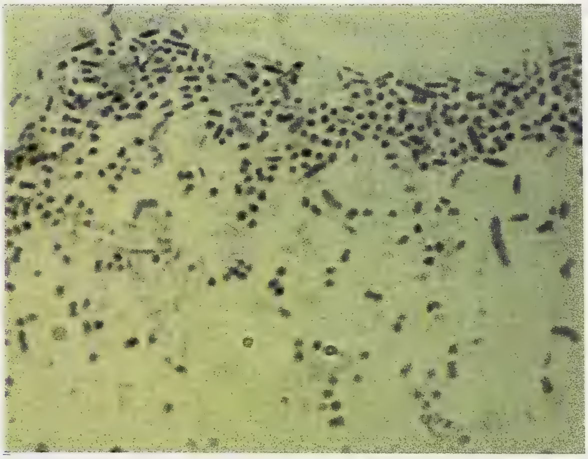
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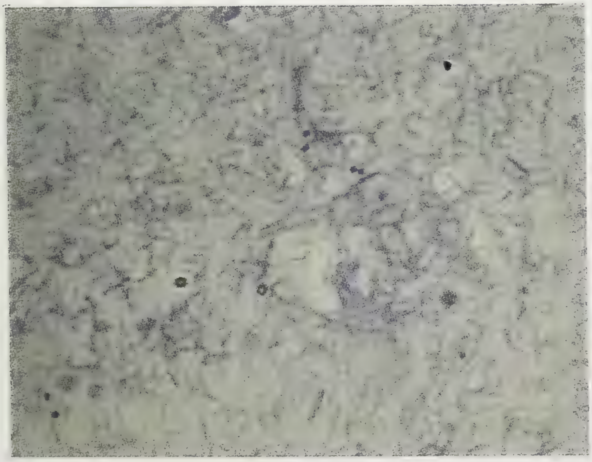


Fig. 5.

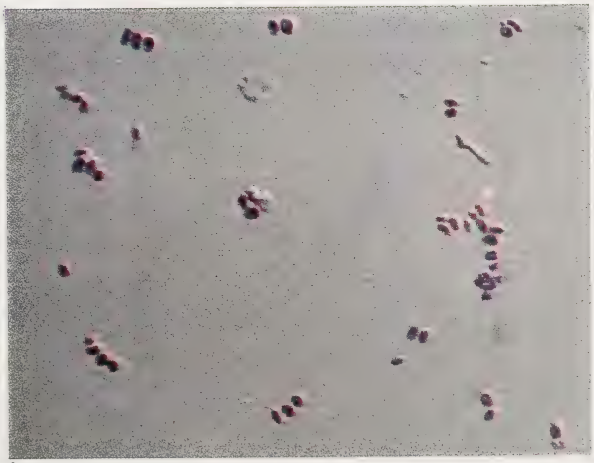


Fig. 6.

10.1.13 Plate 13

Figs. 1-6. Miscellaneous microorganisms found in smeared and stained *Tyrophagus putrescentiae*.

Fig. 1. Round spores of a microsporidian; Source C, batch 4. Stained with Giemsa after methanol fixation (Stain B, method 2).

Fig. 2. As above, Source A, batch 2. Macchiavello stain (Stain E).

Fig. 3. Stirrup-shaped spores similar to those in *A. cucumeris* (see Plate 11, Fig. 1); Source A, Batch 4 (Stain B, method 2).

Fig. 4. Irregular rod-shaped forms similar to those associated with rickettsia in *P. persimilis* (see Plate 6, Figs. 3 and 4); Source C, batch 3 (Stain B, method 2).

Fig. 5. Rods and a few cocci (bacteria?); Source C, batch 4 (Stain E).

Fig. 6. Cluster of unidentified irregular cells, quite common; Source A, batch 4 (Stain B, method 2).

Plate 13

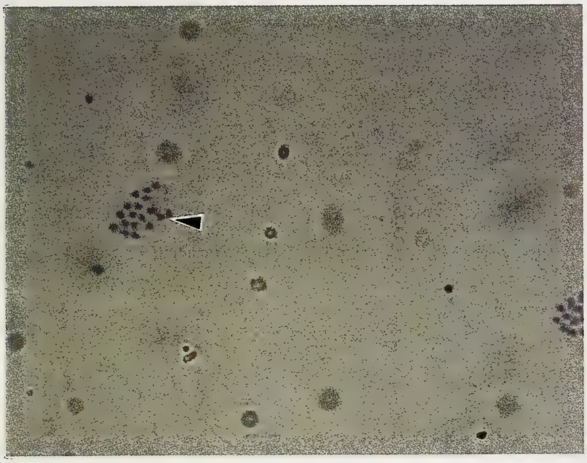
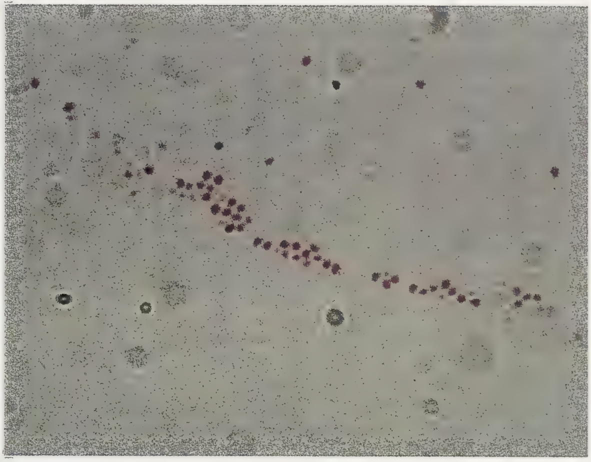


Fig. 1.



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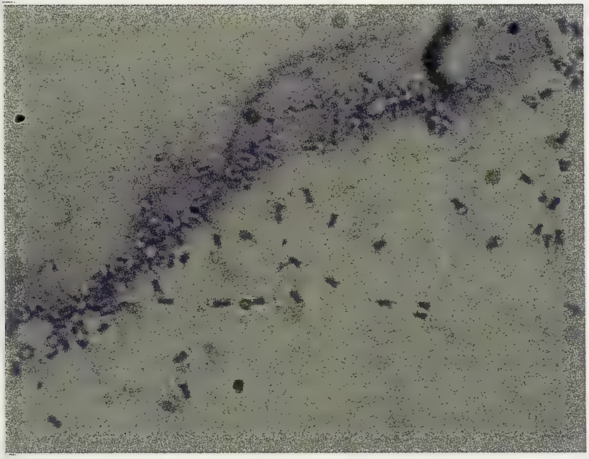

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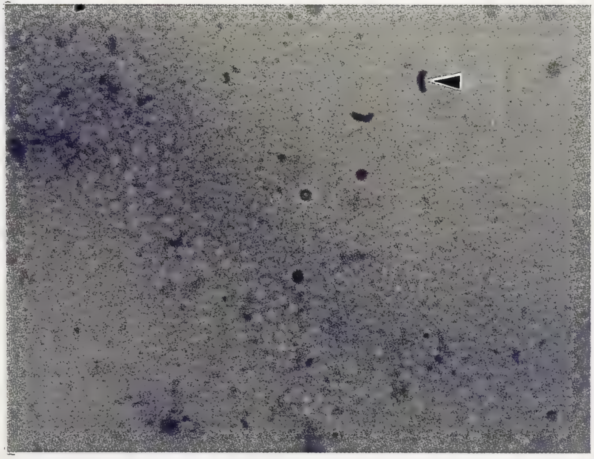


Fig. 4.

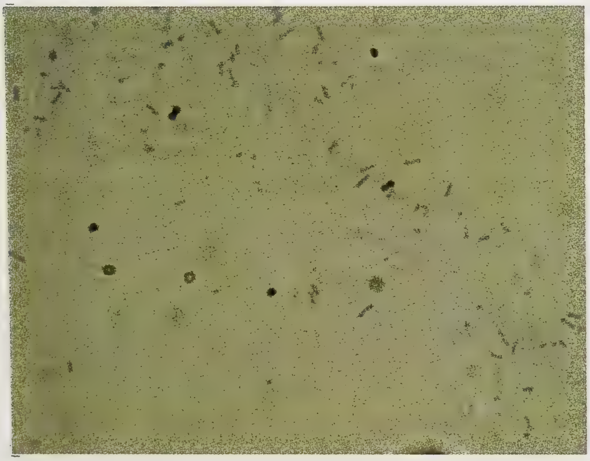


Fig. 5.

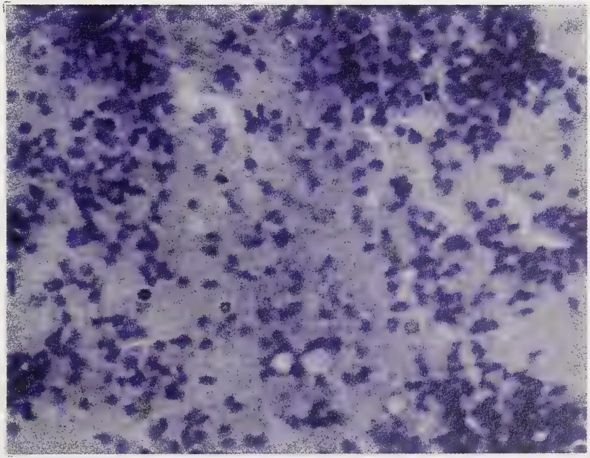


Fig. 6.

10.1.14 Plate 14

Figs. 1-6. Gregarines in smeared and stained *Tyrophagus putrescentiae.*

Fig. 1. Sporozoite stage; Source C, batch 3. Stained with Giemsa after methanol fixation (Stain B, method 2) (Photo courtesy of B.A. Keddie).

Fig. 2. Sporozoite and sporont stages; Source C, batch 3 (Stain B, method 2).

Fig. 3. Sporont stage, as Fig. 2.

Fig. 4. Associated pair of sporonts; Source A, batch 4 (Stain B, method 2).

Plate 14

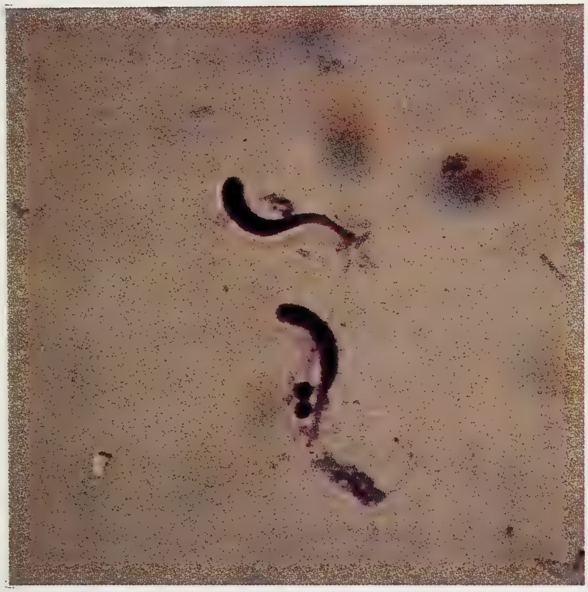


Fig. 1.

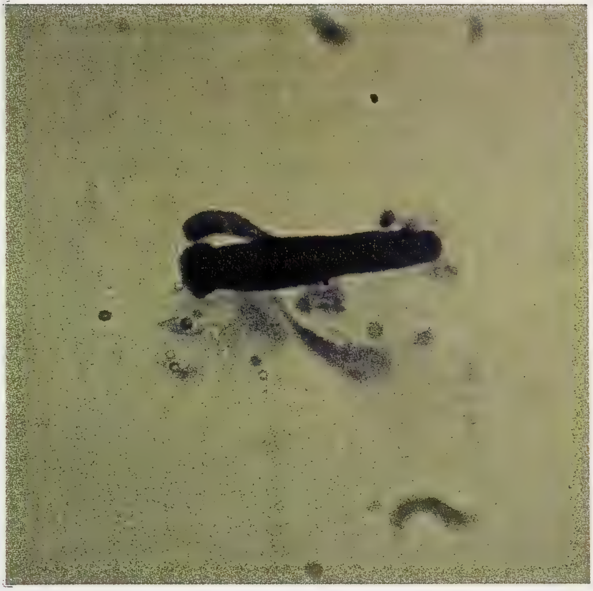


Fig. 2.

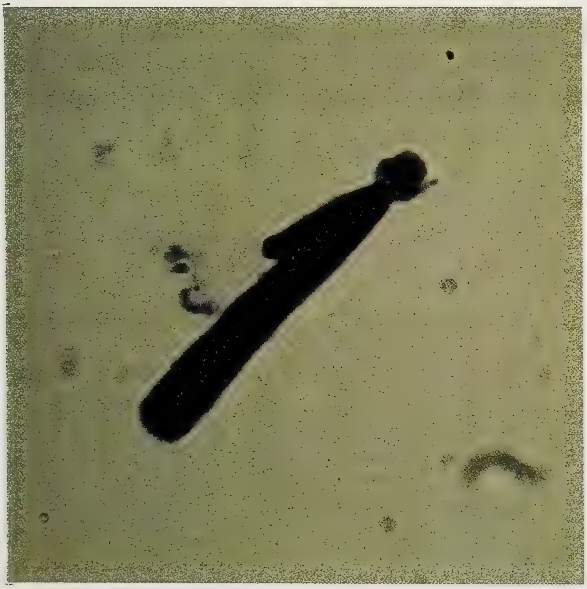


Fig. 3.

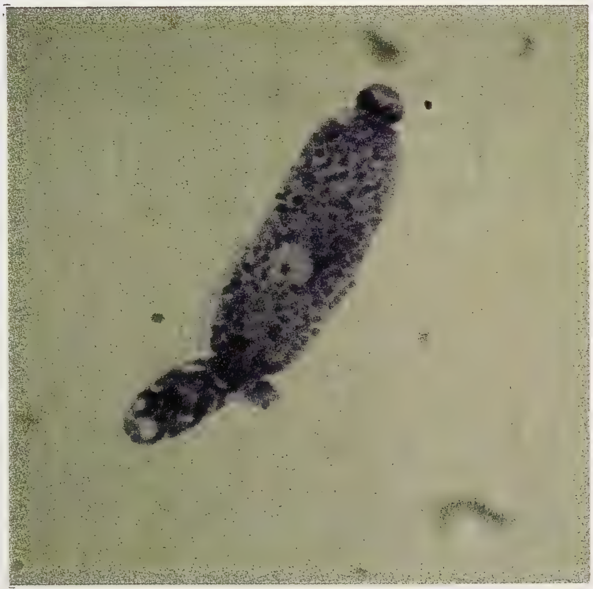


Fig. 4.

10.1.15 Plate 15

Figs. 1-6. Miscellaneous microorganisms found in smeared and stained *Encarsia formosa.*

Fig. 1. Heavy infection with pale-staining coccoid forms; Source C, batch 3 (Stain B, method 2).

Fig. 2. Clump of coccoid forms; Source C; batch 3 (Stain B, method 2).

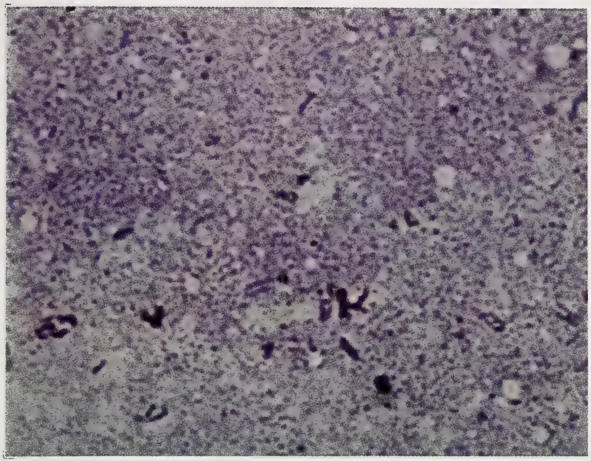
Fig. 3. Egg filled with coccoid forms; Source C, batch 2 (Stain B, method 2).

Fig. 4. Irregular objects, possibly microsporidial stages; Source C, batch 2 (Stain B, method 2).

Fig. 5. As Fig. 4; Source A, batch 1 (Stain B, method 2).

Fig. 6. Unidentified; possibly microsporidian stage; Source C, batch 3 (Stain B, method 2) (x 2,000).

Plate 15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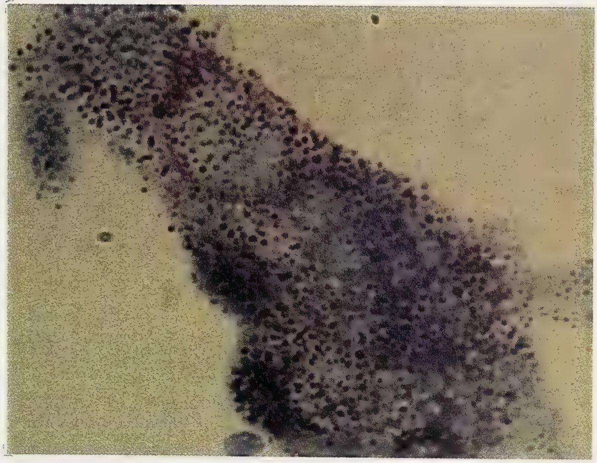
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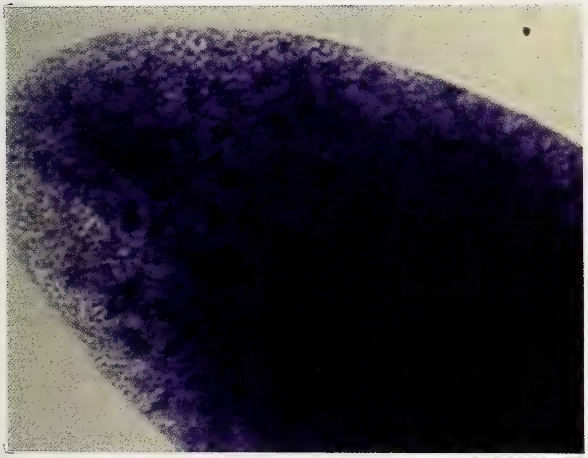
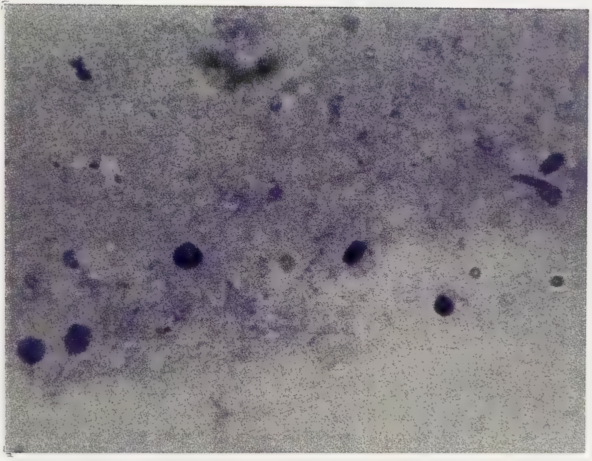


Fig. 3.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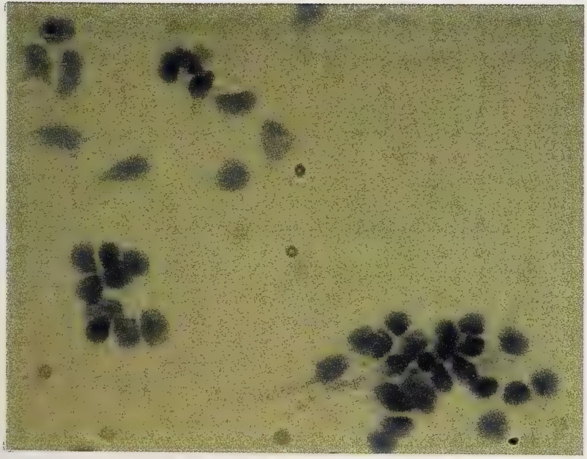
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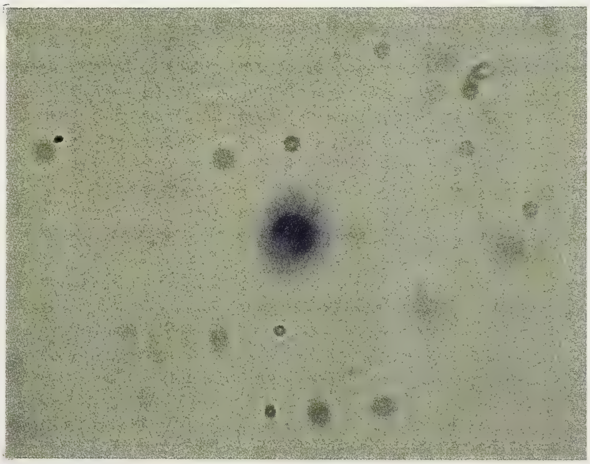


Fig. 6.

10.1.16 Plate 16

Figs. 1-6. Miscellaneous microorganisms and artifacts in smeared and stained *Encarsia formosa* (Figs. 1-5) and *Tetranychus urticae* (Fig. 6).

Fig. 1. Cells infected with rickettsia/bacteria; Source A, batch 3. Stained with Giemsa after methanol fixation (Stain B, method 2). Note apparent infection in nuclei.

Fig. 2. Cell infected with rickettsia/bacteria; Source A, batch 3 (Stain B, method 2).

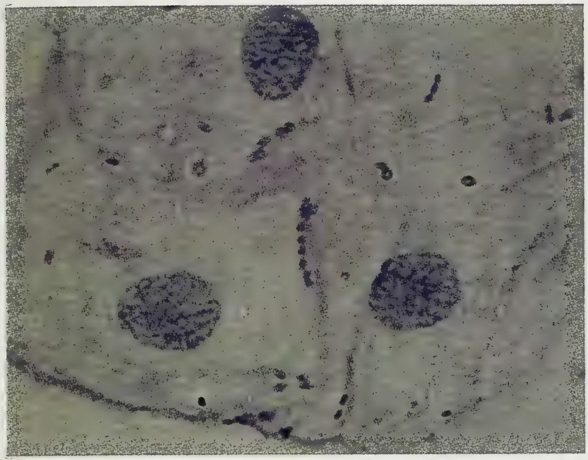
Fig. 3. As Fig. 2, Macchiavello stain (Stain E).

Fig. 4. Crystal formation; Source C, batch 3 (Stain B, method 2).

Fig. 5. Crystal formation; Source A, batch 3 (Stain B, method 2) (see also Plate 8, Fig. 6).

Fig. 6. Mass of coccoid forms in spider mites; original source A (Stain B, method 2).

Plate 16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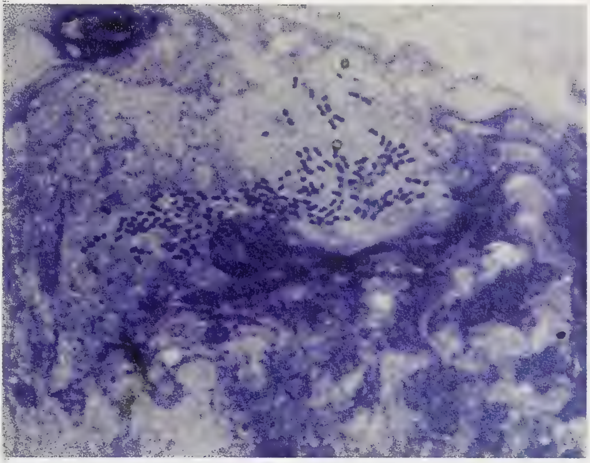
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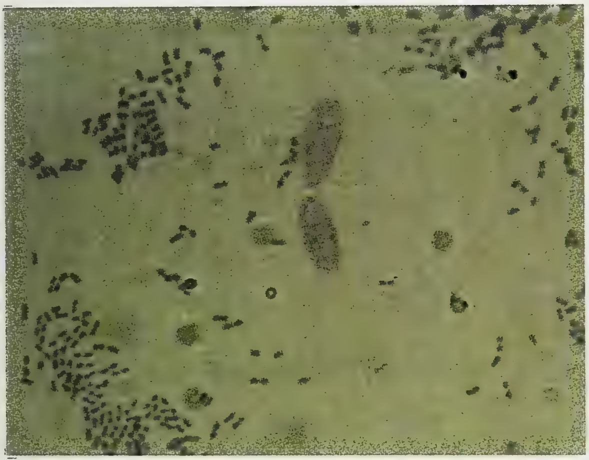
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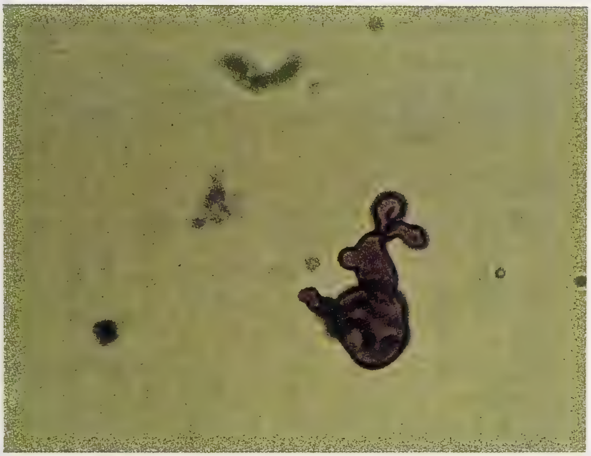
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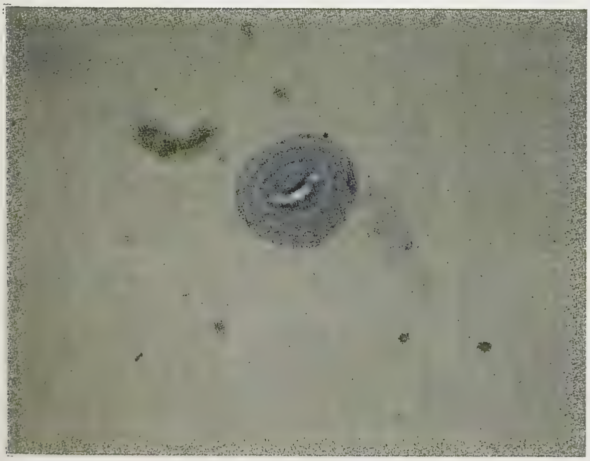
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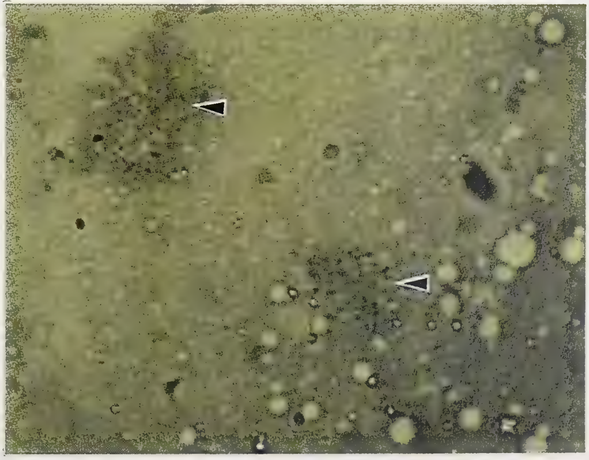


Fig. 6.

10.1.17 Plate 17

Fig. 1. Diagrammatic representation of the digestive system of *Phytoseiulus persimilis* (Chant, 1985. *In*: World Crop Pests. Volume 1B. Spider Mites. Their Biology, Natural Enemies and Control.)

Fig. 2. Diagrammatic representation of a generalized microsporidian spore; APF = anterior portion of coiled polar filament; PPF = posterior portion of polar filament; BPF = basal portion of polar filament; pm = polar sac; P = polaroplast; N = nucleus; OM = outer membrane (exospore); ISM = inner surface membrane (endospore); PM = polar mass and polaroplast membrane; SPO = cytoplasm (Kudo, 1966. *In*: Protozoology, 5th Ed.).

Plate 17

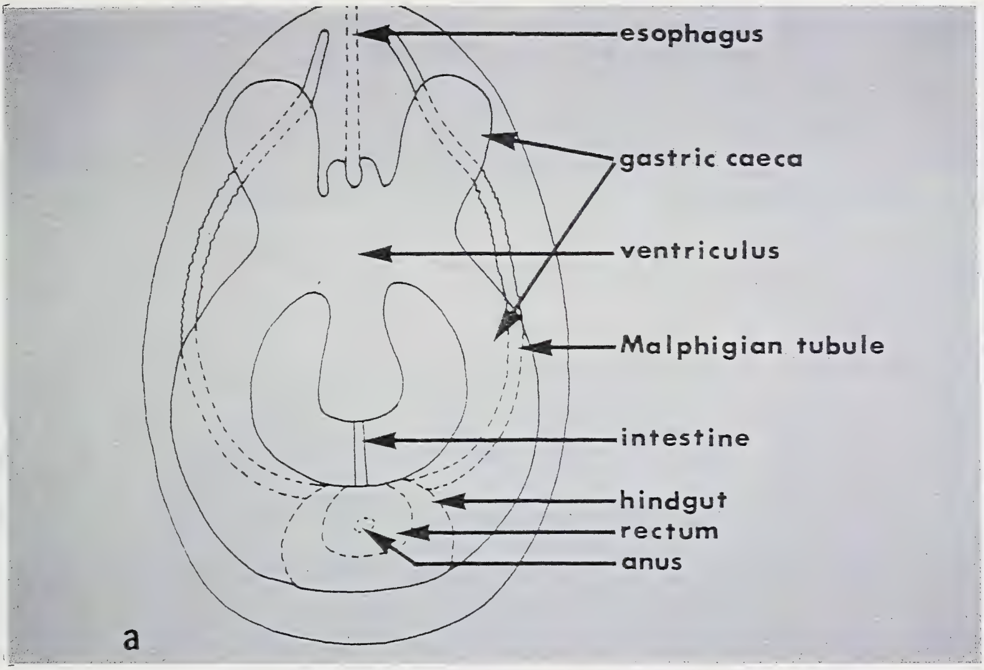


Fig. 1.

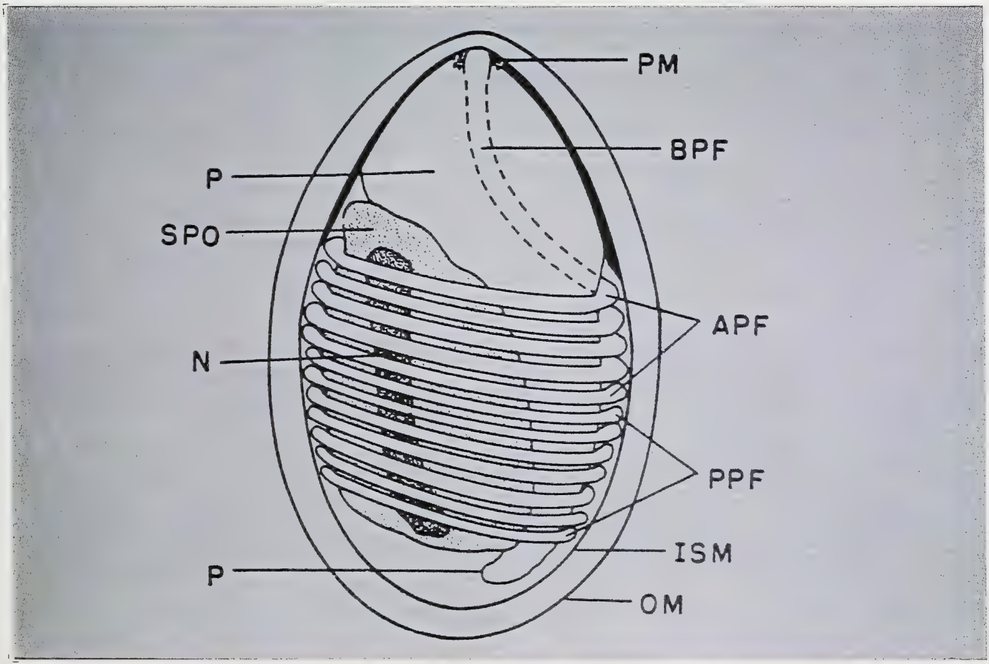


Fig. 2.

10.1.18 Plate 18

Figs. 1-6. Microsporidia found in transmission electron microscopy of *Phytoseiulus persimilis.*

Fig. 1. Spore (rare) Source A (Jan. 1991) (x 23,375).

Fig. 2. Spore, Source A (Jan. 1991) (x 28,125).

Fig. 3. Spore, Source A (Mar. 1991) (x 28,125).

Fig. 4. Spore, Source A (Jan. 1991) (x 33,750).

Fig. 5. Spore of microsporidia, and intracellular rickettsia; Source "Israeli" strain (x 11,250).

Fig. 6. Sporophorous vesicle containing spores and sporogonial stages; Source A (Dec. 1990) (x 6,875).

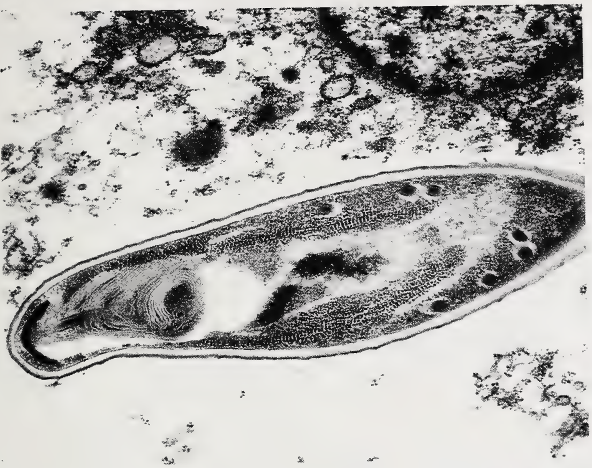


Fig. 1.

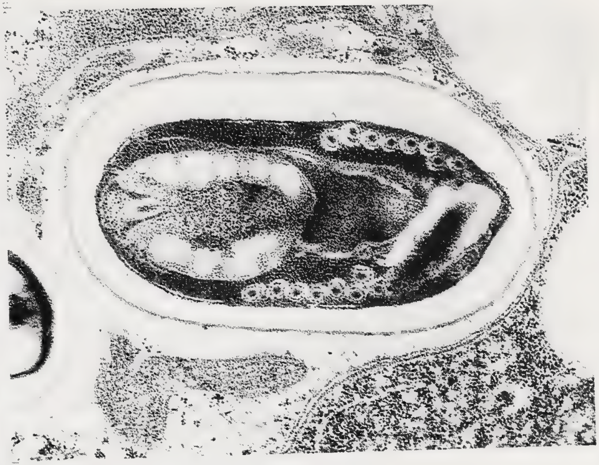
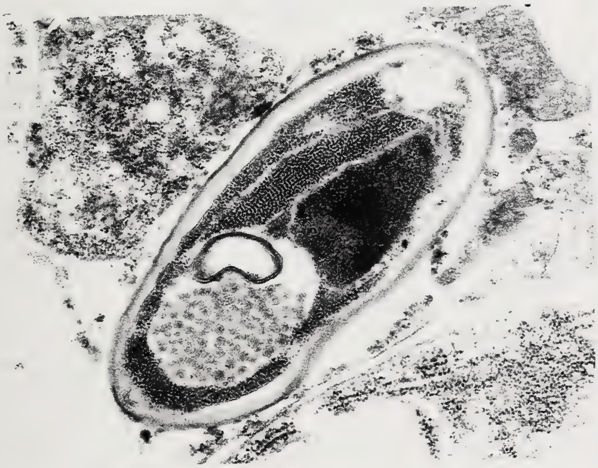


Fig. 2.



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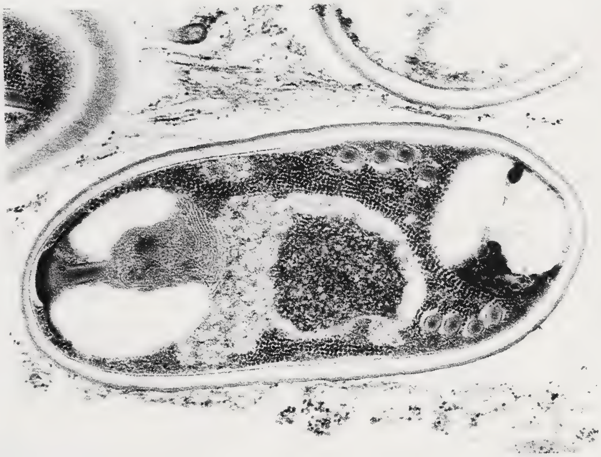
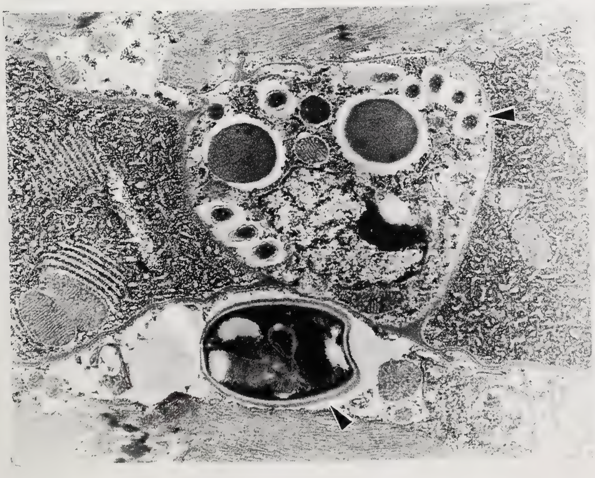


Fig. 4.



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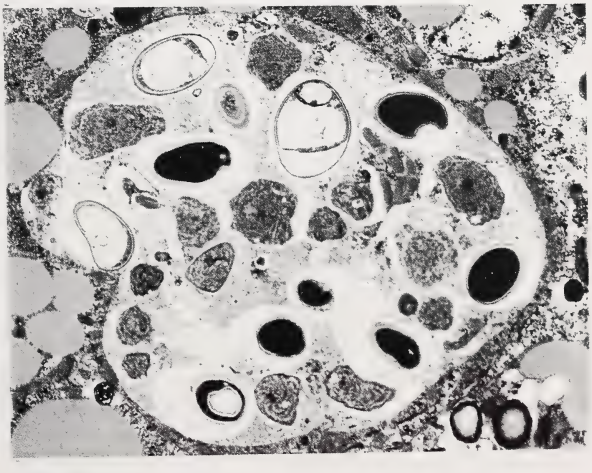


Fig. 6.

10.1.19 Plate 19

Figs. 1-6. Transmission electron micrographs of microsporidia in *P. persimilis* and *T. putrescentiae*.

Fig. 1. Microsporidian spores in cytoplasm of *P. persimilis* and wall of intestine; Source A, batch 1 (x 1,500).

Fig. 2. Microsporidian spores and sporoblasts (lower and upper right); fungal spore (far left), calcium crystals (centre) and unidentified microorganisms in *P. persimilis*; Source "Israeli" strain (x 3,125).

Fig. 3. Microsporidian spores (centre) and melanized objects in *P. persimilis*; Source A, batch 4 (x 6,250).

Fig. 4. Round microsporidian spores (lower left) and rickettsia in *Tyrophagus putrescentiae*; Source C, batch 2 (x 5,625).

Fig. 5. Round microsporidian spores in sporophorous vesicle in *Tyrophagus putrescentiae*; Source C, batch 2 (x 13,500).

Fig. 6. Sporonts and sporoblasts developing into spores in *Tyrophagus putrescentiae*; Source C, batch 2 (x 11,250).

Plate 19

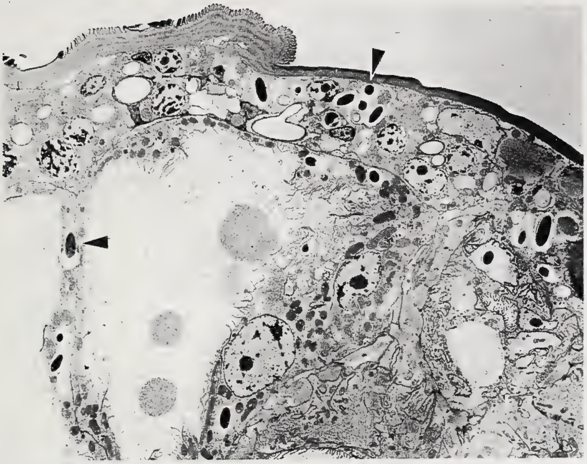
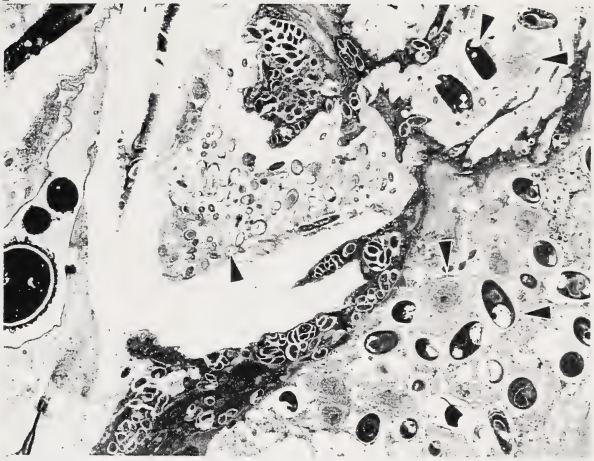


Fig. 1.



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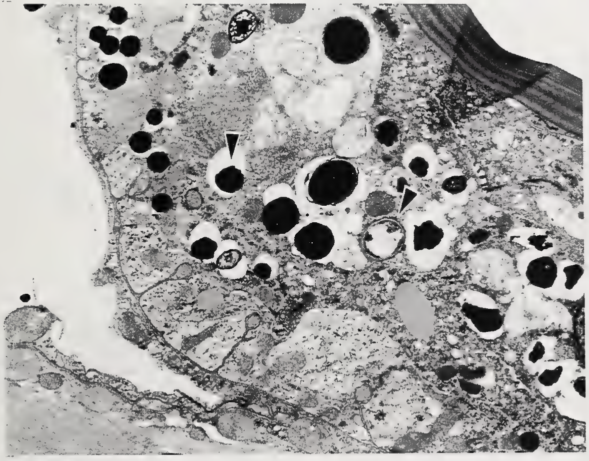

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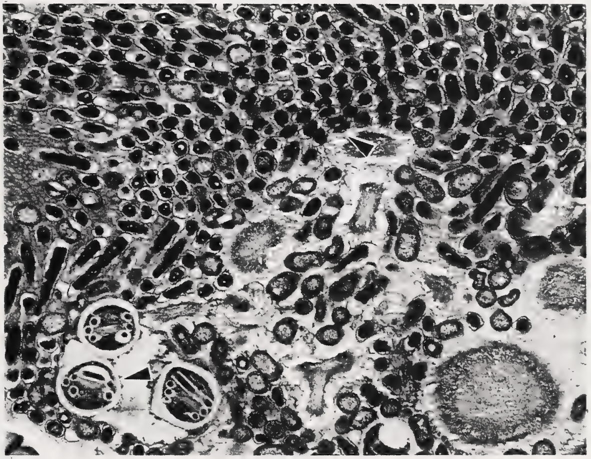

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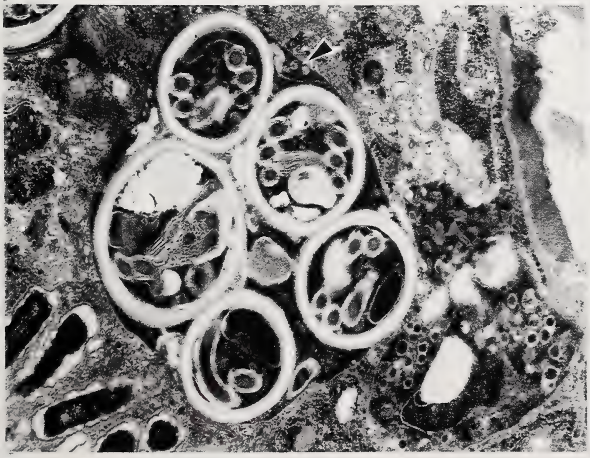

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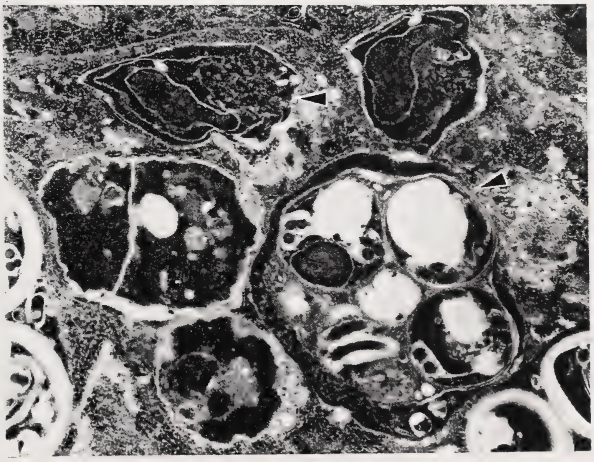


Fig. 6.

10.1.20 Plate 20

Figs. 1-6. Transmission electron micrographs of various microsporidia and artifacts.

Fig. 1. Microsporidian spore within developing embryo of *P. persimilis*; Source A (Mar. 1991) (x 6,250).

Fig. 2. Round microsporidian spore and rickettsia after discharge through anus of *T. putrescentiae* (right); Source C, batch 2 (x 3,125).

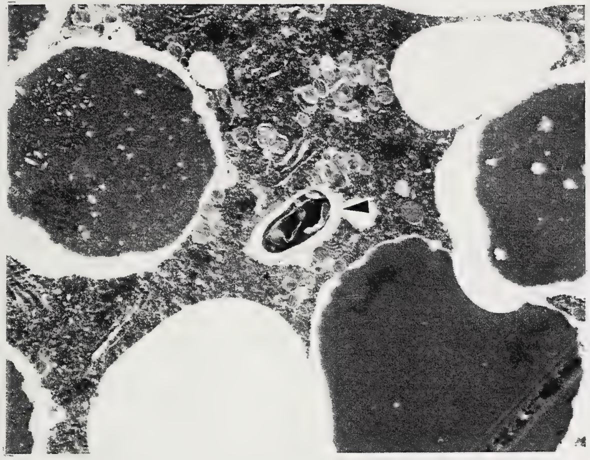
Fig. 3. Calcium crystals causing "white gut" symptoms in *P. persimilis*; Source A, batch 3 (x 6,250).

Fig. 4. Calcium crystals in digestive tract of *A. cucumeris*; Source C, batch 2 (x 6,250).

Fig. 5. Calcium crystals within cytoplasm in *P. persimilis*; Source B, batch 4 (x 7,500).

Fig. 6. Calcium crystals (left) and rickettsia in broken-down tissues of *P. persimilis*; Source C, batch 2 (x 4,375).

Plate 20



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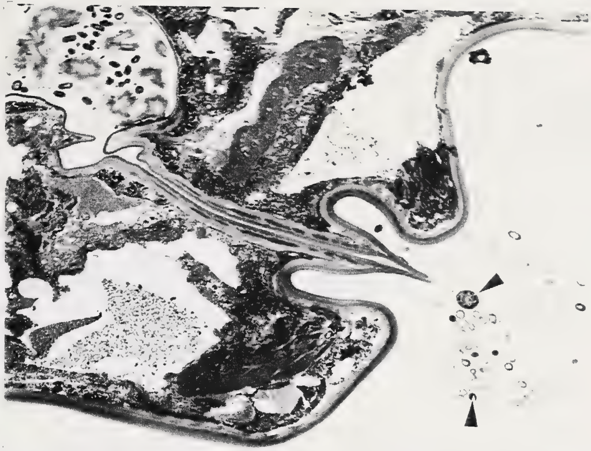

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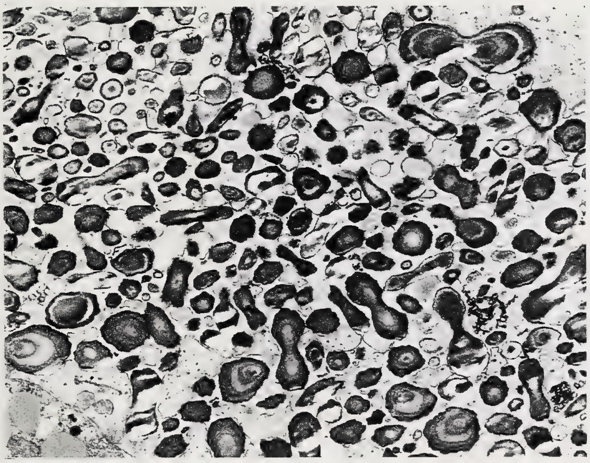

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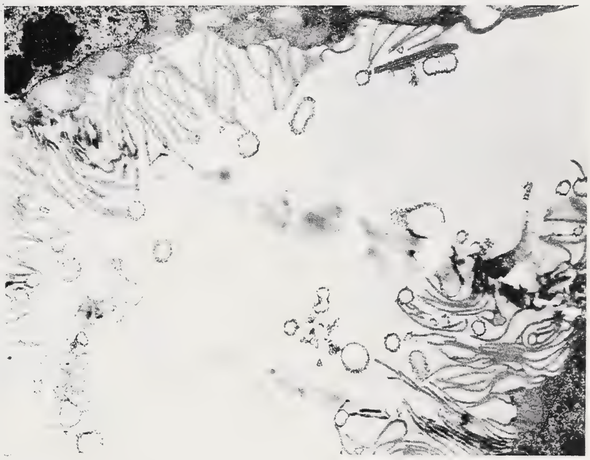

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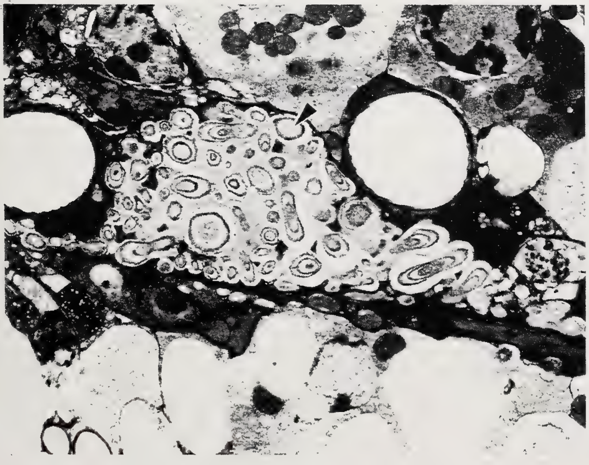

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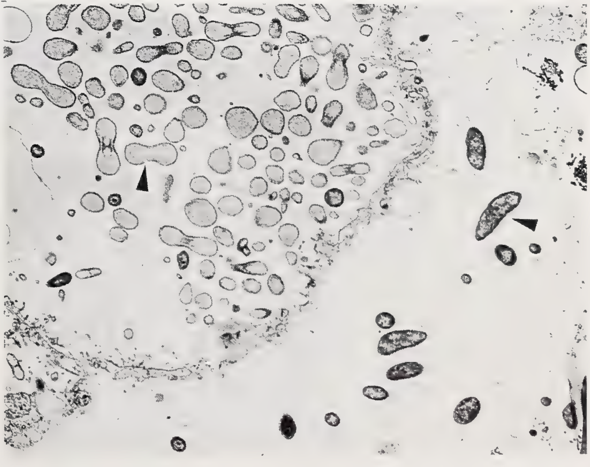


Fig. 6.

10.1.21 Plate 21

Figs. 1-6. Transmission electron micrographs of rickettsia in *P. persimilis.*

Fig. 1. Extensive rickettsial infection. Note dark crystals; Source C (from cages) (x 2,250).

Fig. 2. Rickettsial stages, primarily bacterial rickettsia, with possibly intermediate and giant forms also present; Source A, batch 10 (x 5,625).

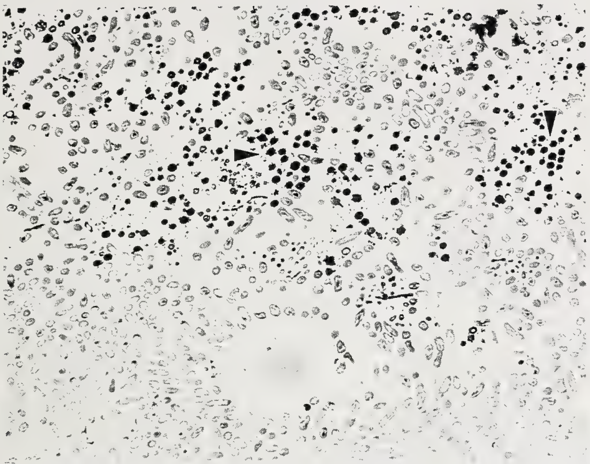
Fig. 3. Dense and bacterial rickettsia; Source A, batch 3 (x 6,250).

Fig. 4. Dense rickettsia; Source A, batch 8 (x 22,500).

Fig. 5. Bacterial rickettsia; Source A, batch 6 (x 22,500).

Fig. 6. Irregularly-shaped bacterial rickettsia; Source C, batch 6 (in legs) (x 22,500).

Plate 21



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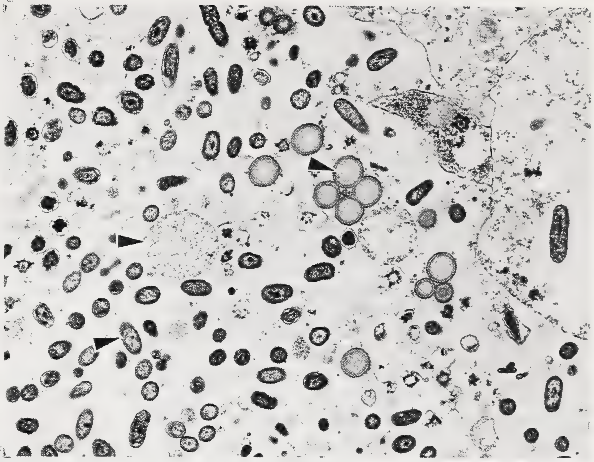

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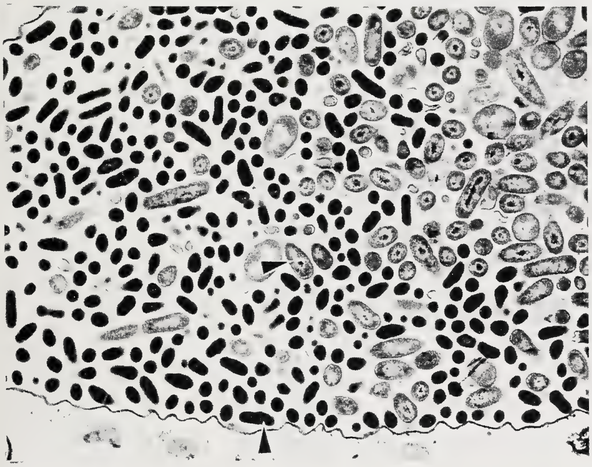

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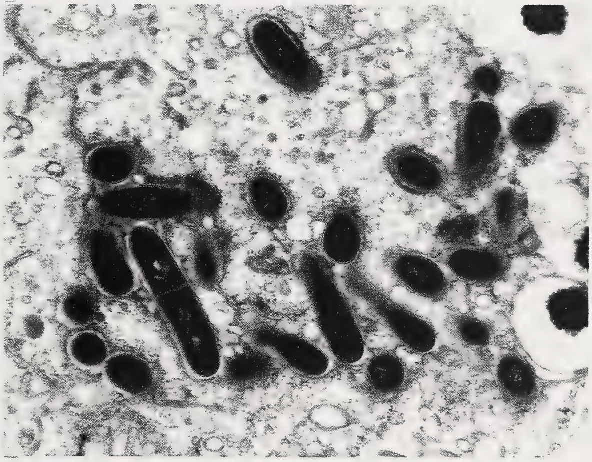

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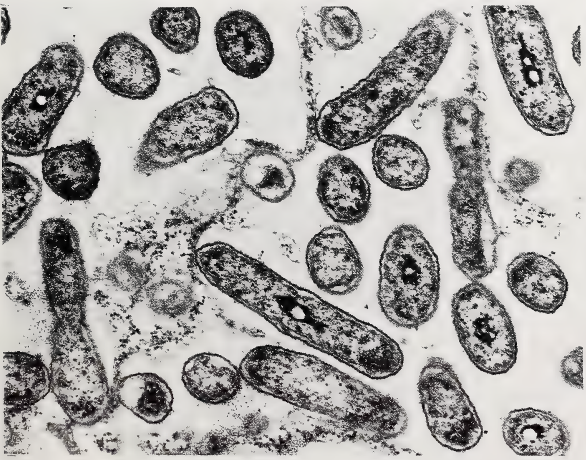

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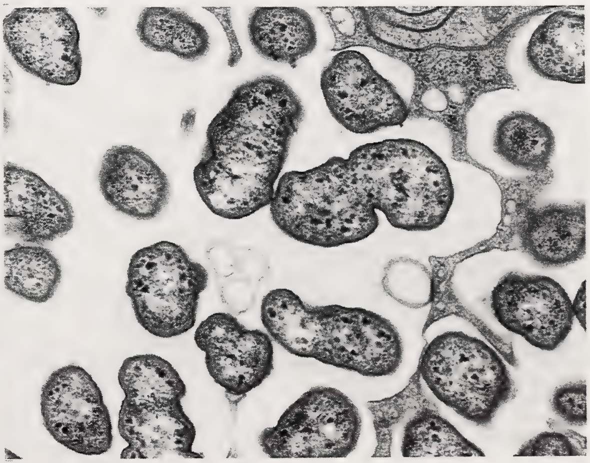


Fig. 6.

10.1.22 Plate 22

Figs. 1-6. Transmission electron micrographs of various artifacts.

Fig. 1. Dense rickettsia, *P. persimilis*; Source B, batch 1 (x 13,500).

Fig. 2. Vacuole containing rickettsia, *T. urticae*, Source A (x 11,250).

Fig. 3. Rickettsia (upper centre) in association with melanized objects in *P. persimilis*; Source B, batch 1 (x 5,625).

Fig. 4. Close up of rickettsia in Fig. 3, showing cell wall around some melanized objects (centre and upper left) (x 16,875).

Fig. 5. Unidentified structures in *E. formosa* containing crystal-like artifacts; Source C, batch 3 (x 5,625).

Fig. 6. Unidentified artifact in *P. persimilis*; Source B, batch 8 (x 33,750).

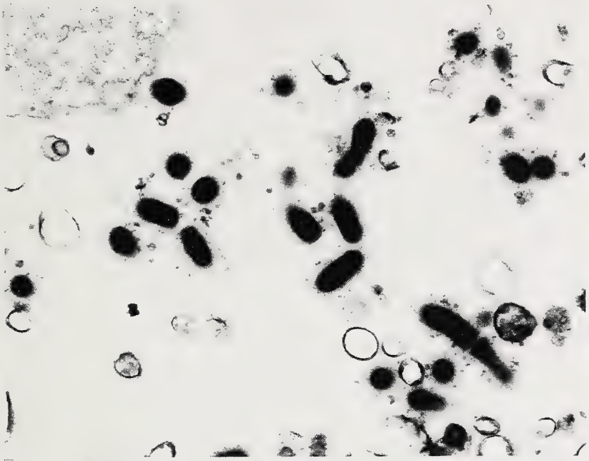
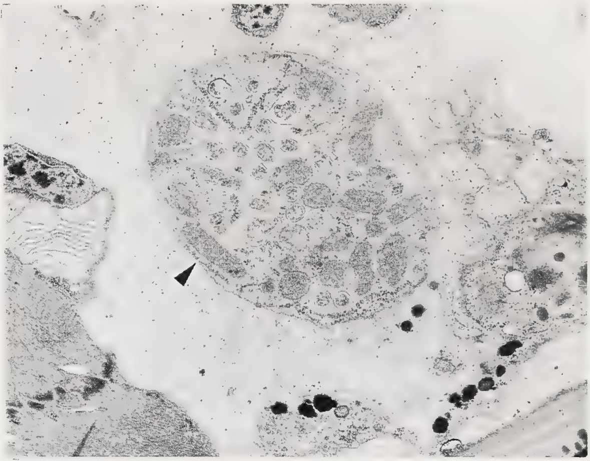


Fig. 1.



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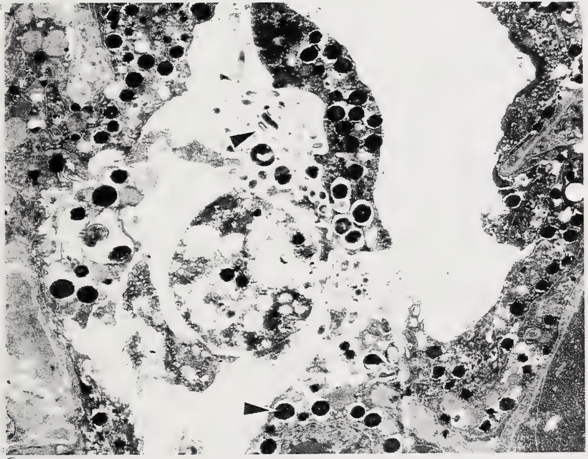

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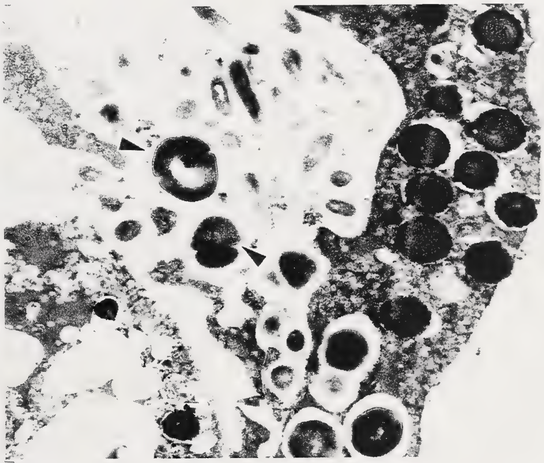

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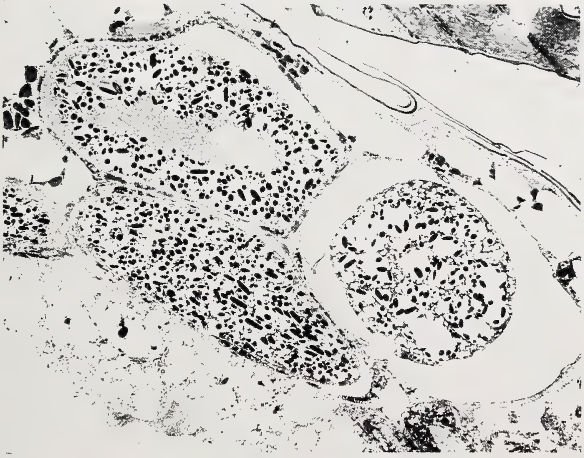


Fig. 5.

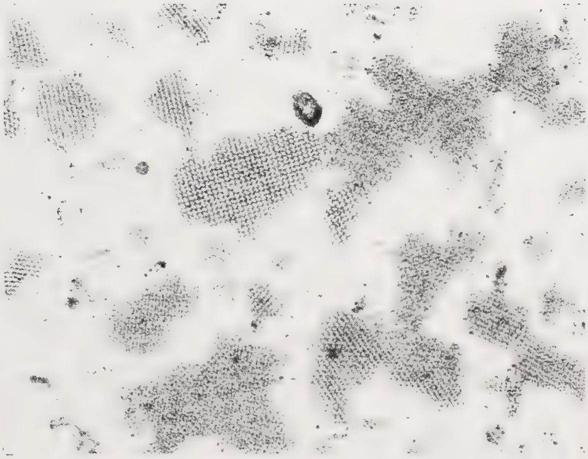


Fig. 6.

10.1.23 Plate 23

Figs. 1-6. Transmission electron micrographs of virus-like particles.

Fig. 1. Non-occluded virus particles in embryo of *A. cucumeris*; Source C, batch 4 (x 11,250).

Fig. 2. Non-occluded virus particles in embryo of *P. persimilis*; Source A (Dec. 1992) (x 5,625).

Fig. 3. Non-occluded virus particles in embryo of *P. persimilis*; Source A (from cages) (x 18,750).

Fig. 4. Non-occluded virus particles in embryo of *T. putrescentiae*; Source A, batch 3 (x 9,000).

Fig. 5. Non-occluded virus particles in embryo of *A. cucumeris*; Source A (Jun. 1991) (x 18,750).

Fig. 6. Non-occluded virus particles in embryo of *E. formosa*; Source A, batch 1 (x 5,625).

Plate 23

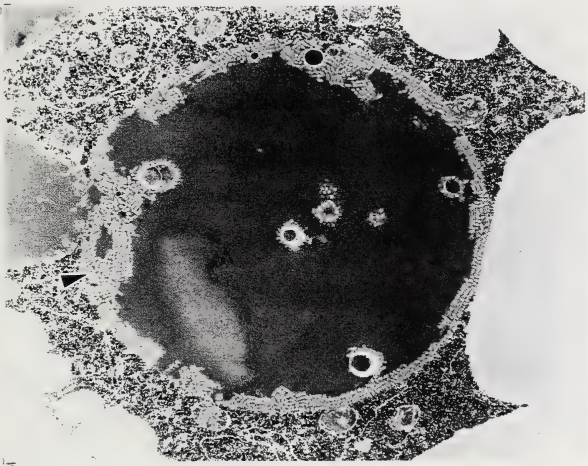
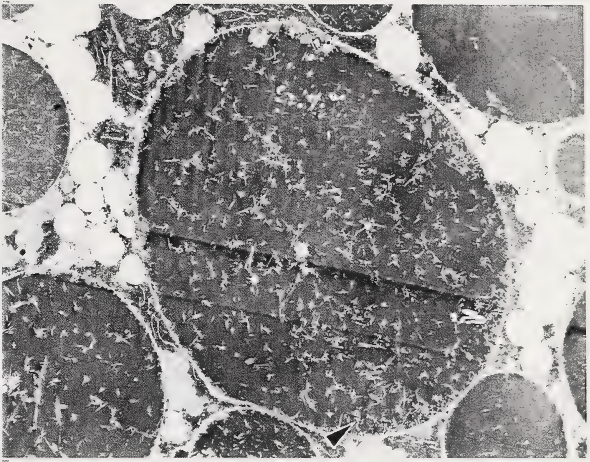


Fig. 1.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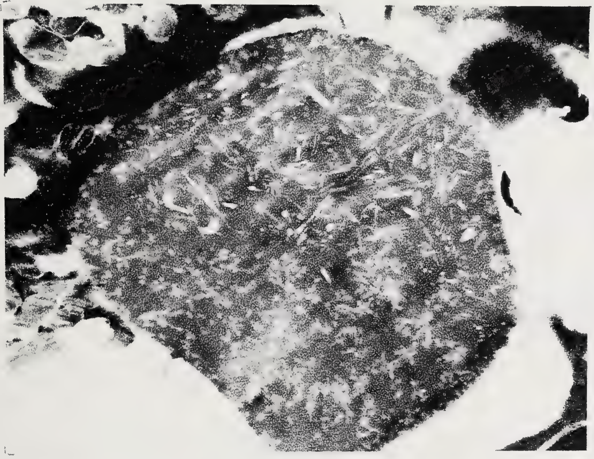
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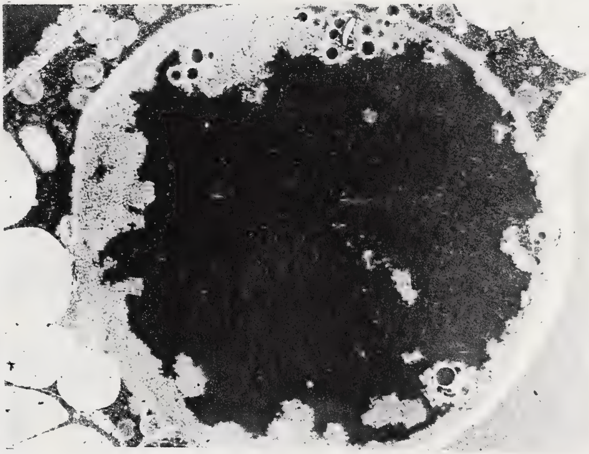
Fig. 4.

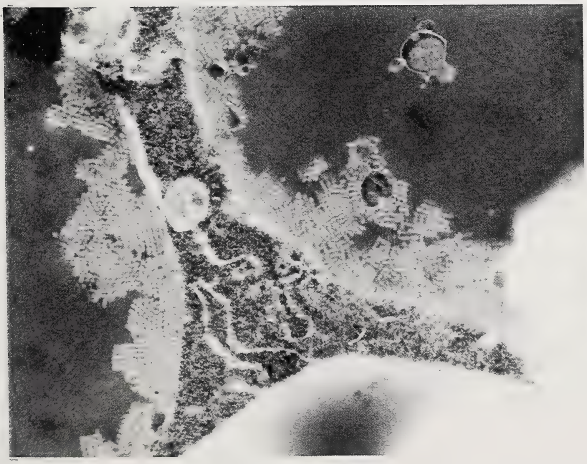
Fig. 5.

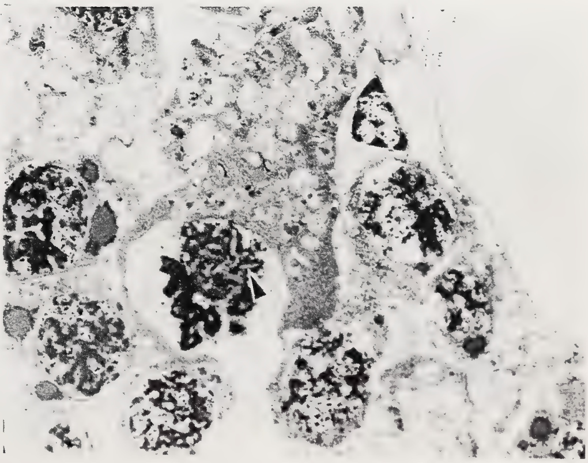
Fig. 6.

11 APPENDIX IV

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